AMMONIUM SULPHATE ENHANCEMENT OF PICLORAM HERBICIDAL ACTIVITY AND ABSORPTION IN TWO GUAVA SPECIES AND DWARF BEANS

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ABSTRACT

Studies were conducted to increase picloram herbicidal activity on guava (Psidium guajava L.), strawberry guava (Psidium cattleianum Sabine) and dwarf beans (Phaseolus vulgaris L. cv. Bountiful) using ammonium sulphate as an adjuvant, and to determine the basis for the enhancement effect. Guava and strawberry guava seedlings were grown in pots outdoors for 3 to 4 months and 4 to 5 months respectively, and subsequently in the glasshouse for 2 months, before treatment with picloram alone at 0.25, 0.50 and 0.75 kg a.i./ha, or plus ammonium sulfate at 0.10, 0.75, 1.0 and 10% (w/v). Dwarf beans were treated with picloram alone at 0.02 and 0.04 kg a.i./ha, or plus ammonium sulfate at 0.10, 0.50, 1.0, 5.0 and 10% (w/v). Ammonium sulfate increased picloram herbicidal activity in all three species to varying degrees, depending on picloram rate and ammonium sulfate concentration. Picloram injury was enhanced by ammonium sulfate at all picloram rates shortly after treatment. The higher picloram rates continued to increase injury with time, so that by the end of the experiment on each species, the maximum effect was attained without ammonium sulfate and an enhancement effect could not be demonstrated. The enhancement effect remained evident at the low picloram rates. Ammonium sulfate concentrations of 0.75% to 1.0% and higher were equally effective at increasing picloram activity at the end of each experiment, but at earlier times, picloram activity increased

with increasing ammonium sulfate concentration up to 10%.

Paired leaves on the upper stem of strawberry guava and guava seedlings were treated with ^{14}C-picloram alone or plus ammonium sulfate at 0.5% and 10% (w/v) on strawberry guava, and 0.5% on guava. The ^{14}C-picloram acid was converted to the potassium salt using commercial formulation blank, so that the ^{14}C solutions were equivalent to the picloram solutions used in the activity experiments. After 2 days on strawberry guava, 0.5% ammonium sulfate increased ^{14}C-picloram absorption about five-fold. There was about four times more ^{14}C in the upper stem and attached leaves with ammonium sulfate added than with picloram alone. ^{14}C-picloram absorption from 0.5% and 10% ammonium sulfate treatments was equal. Ammonium sulfate at 0.5% increased picloram absorption by four-fold in guava, but there was no increase in the amount of ^{14}C translocated. All the ^{14}C in both guava species was shown to be ^{14}C-picloram by paper chromatography of extracts. Picloram absorption by dwarf beans, measured by difference between the amount of picloram applied and the amount recovered, was increased by 0.5% and 10% ammonium sulfate by a similar magnitude as on guava species.

Picloram absorption was further studied using detached strawberry guava leaves, and guava leaves in an initial experiment. ^{14}C-picloram absorption was greater through the lower surface than through the upper surface, with or without ammonium sulfate. In strawberry guava leaves, ^{14}C-picloram absorption through the upper and lower leaf surfaces was

was equal, but the ammonium sulfate enhancement of absorption was much greater through the upper surface. Ammonium sulfate increased the rate of ^{14}C-picloram absorption, but absorption from ^{14}C-picloram alone or plus ammonium sulfate levelled off at 6 to 12 hours. The magnitude of the ammonium sulfate-induced increase in ^{14}C-picloram absorption was not affected by picloram concentration in the range 250 to 2000 ppmw, or by leaf age. ^{14}C-picloram absorption by this detached leaf method was highest at an intermediate ammonium sulfate concentration range, such that absorption with 0.1% and 10% ammonium sulfate was less than with 0.5%; 0.01% had no effect on absorption.

Increased ^{14}C-picloram absorption resulted from adding the ammonium salts of sulfate, nitrate, chloride or dibasic phosphate, but not from ammonium carbonate or ammonium molybdenate. Ammonium sulfate was the only one of six sulfate salts tested to increase picloram absorption.

pH adjustment of the external solution from 6 to 4 increased picloram absorption, while a pH change from 6 to 8 had no effect. In buffered solution at pH 4 ammonium sulfate did not increase ^{14}C-picloram absorption. Ammonium dibasic phosphate at a solution pH of 7.7 increased picloram absorption as effectively as ammonium monobasic phosphate at pH 4.6. Potassium monobasic phosphate at pH 4.6 increased absorption to about one-half the value with ammonium phosphates added, whereas potassium dibasic phosphate at pH 9.5 had no effect.

Stomata are not present on the upper surface of

strawberry guava leaves, thus in the measurement of the effect of ammonium sulfate on picloram absorption, cuticular penetration of picloram must have been involved. The evidence suggests that ammonium sulfate has a direct physical effect in the absorption pathway.

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CHAPTER I

INTRODUCTION

Guava (Psidium guajava L.) is both a tropical fruit crop and a serious weed in tropical areas (Cates 1967; Mootooka et al. 1967a; Tschirley et al. 1967). Control of guava by conventional mechanical methods is both uneconomical and physically non-feasible on steep mountainsides. Furthermore guava is a prolific producer of sprouts following cutting or other mechanical disturbance (Tschirley et al. 1967). An effective and economical method of chemical control is needed, involving aerial application of herbicides on inaccessible lands. The most effective herbicides available do not give complete control, and in addition high herbicide rates must be used (Bovey et al. 1969; Dowler and Tschirley 1970; Plucknett 1969; Tschirley 1968; Tschirley et al. 1967). High rates are undesirable from both the environmental and economical aspect. Strawberry guava (Psidium cattleianum Sabine) presents a similar serious problem, though it is not so widespread as guava (Plucknett 1969).

One way to improve control of the guava species is to attempt to increase the activity of the herbicide known to be most effective. The objectives of this study were: (1) to enhance herbicide activity on both guava species with the use of an inorganic salt adjuvant, (2) to examine the effect of a successful salt adjuvant on herbicide absorption and translocation, as a means of explaining the enhancement

effect, and (3) to study the factors affecting enhanced absorption as leads to an understanding of the mechanism involved.

CHAPTER II

REVIEW OF LITERATURE

Guava (Psidium guajava L.) is a tree or shrub with a pan-tropical distribution. It is a serious weed on ranchland and coconut plantations in tropical areas such as Hawaii, Puerto Rico and Fiji (Cates 1967; Motooka et al. 1967; Tschirley et al. 1968). Guava has been declared a noxious weed in Hawaii (Anon. 1962). Guava is a weed of major importance because it spreads rapidly through seed dissemination and is difficult to control with herbicides (Tschirley et al. 1967). Strawberry guava (Psidium cattleianum Sabine) is also a weed on ranchland and also very difficult to control (Plucknett 1969).

Chemical Control of Guava

The most effective herbicide for long term defoliation of guava is the potassium salt of 4-amino-3,5,6-trichloropicolinic acid (picloram) (Tshirley 1968). It was more effective than the dimethylamine salt of 3,6-dichloro-o-anisic acid (dicamba), which was in turn more effective than 2,4-dichlorophenoxyacetic acid (2,4-D), 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), and 2-(2,4,5-trichlorophenoxy) propionic acid (silvex) (Bovey et al. 1969; Dowler and Tschirley 1970; Plucknett 1969; Tshirley 1968; Tschirley et al. 1967).

Contact herbicides such as 1,1'-dimethyl-4,4'-

bipyridinium salt (paraquat) and 6,7-dihydrodipyrido (1,2-a:2',1'-c) pyrazinediium dibromide salt (diquat) cause rapid defoliation of guava but are ineffective for long term control (Motooka et al. 1967 a; Tschirley 1968).

Picloram has generally resulted in 65% to 95% defoliation of guava by one year after treatment (Bovey et al. 1969; Tschirley 1968; Tschirley et al. 1967), although picloram has produced 100% defoliation, provided the guava was treated during the wet season (Dowler and Tschirley 1970). In some cases, regrowth of guava has commenced by one year after treatment (Bovey et al. 1969; Tschirley 1968). Observations on strawberry guava wherever it was present in trial plots, indicate that it may be even more difficult to control than guava (Plucknett, personal communication). One way to improve control of both guava species is to increase picloram activity. Additionally, picloram is more persistent in the soil than other growth regulator-type herbicides (Goring et al. 1965; Keys and Friesen 1968) so that it is desirable to use rates as low as possible. Thus an increase in picloram activity might permit a high degree of guava control with desired lower rates of picloram.

Herbicide spray solution adjuvants usually increase herbicide activity while some may be used to reduce spray drift. These substances include surfactants, oils, humectants, film-forming substances, thickening agents, growth regulators, penetrants, and inorganic salts (Hull 1970). Inorganic salts have markedly increased the activity of several

herbicides so the use of inorganic salts as adjuvants may be one way to increase picloram activity. A very recent report now indicates that ammonium salts do increase picloram activity on guava (Turner and Loader 1972).

Effect of Inorganic Salts on Herbicidal Activity

Ammonium salts have been more widely tested than other inorganic salts and found to be equally or more effective in increasing herbicide activity. Ammonium salts, have commonly been reported to increase 2,4-D activity. For instance the control of Russian thistle (Salsola kali L.) by 2,4-D amine was increased from a rating of 1.0 (0-10 scale) to 6.0 with ammonium phosphate added, to 5.5 with ammonium sulfate and 4.2 with ammonium nitrate (Sexsmith 1953). For the control of perennial sowthistle (Sonchus arvensis L.) and knotweed (Polygonum aviculare L.), the toxic action of 0.5% 2,4-D Na salt plus 10% ammonium nitrate was equal to 1.0 - 1.5% 2,4-D alone (Al'tergot and Kiselev 1963). Other reports indicate that ammonium salts, primarily sulfate and nitrate, cause similar increases in the control of annual weeds with 2,4-D (Borodna et al. 1962; Chesalin 1962; Ladonin 1958, 1961; Likholat 1962; Lobarchuk 1962; Makodzeba 1962; Shcheglov 1962; Wort 1962; Zambin 1962).

Ammonium salts also enhanced the activity of other herbicides, such as picloram (Turner and Loader 1972) the salt of 2-methyl-4-chlorophenoxyacetic acid (MCPA salt)

(Turner and Loader 1972), monosodium methane arsenate (MSMA), sodium salt of 2,2-dichloropropionic acid (dalapon), and isopropylamine salt of N-(phosphonomethyl) glycine (glyphosate) (Wills 1971, 1973). Picloram activity on seedlings of guava was enhanced by ammonium sulfate, ammonium nitrate, ammonium chloride and ammonium citrate (Turner and Loader 1971). Ammonium sulfate was the most effective salt, reducing the weight of new growth on guava to 1.1 g from 10.6 g for picloram alone. Ammonium nitrate produced a similar marked enhancement of the activity of MCPA salt on willow (Salix fragilis L.) and popular (Populus gelrica Ait.) (Turner and Loader 1972). The control of purple nutsedge (Cyperus rotundus L.) by MSMA, dalapon and glyphosate was increased by a series of ammonium salts, with phosphate being the most effective (Wills 1971, 1973).

Herbicide activity may not be increased by all ammonium salts, as indicated by results with the growth regulator sodium salt of naphthalenlacetic acid (NaNAA). Horsfall and Moore (1962) reported that the increase in apple leaf angle curvature produced by NaNAA was not affected by ammonium borate, molybdate and acetate, while the angle was reduced by adding ammonium carbonate and ammonium bicarbonate. However the activity of NaNAA was increased by other ammonium salts: ammonium bisulfate, ammonium chloride, ammonium nitrate, ammonium sulfate, ammonium monobasic phosphate and ammonium dibasic phosphate.

Ammonium thiocyanate is reviewed separately from other

ammonium salts because its mode of action and range of effective concentration appear to differ. Also it is phytotoxic at much lower concentrations than other ammonium salts (Turner and Loader 1962). Ammonium thiocyanate is used commercially as an adjuvant in the product Amitrole-T, where it is added to 3-amino-s-triazole (amitrole). Amitrole effectiveness is generally increased two to four times by ammonium thiocyanate (Crafts and Robbins 1962; Holly and Chandler 1960; Sterry 1961).

The effect of ammonium thiocyanate on 2,4,5-T activity has been investigated on a number of brush species. Defoliation of winged elm (Ulmus alata Michx.) by 2,4,5-T ester in a diesel oil in water emulsion was increased by ammonium thiocyanate from 33% to 93% (Elwell 1968). Also, Robison (1965) found an increase in canopy reduction of mesquite (Prosopis juliflora (Sw.) DC.) from adding ammonium thiocyanate. However, Tschirley (1968) reported that ammonium thiocyanate did not affect 2,4,5-T ester activity on winged elm, and that it reduced activity on mesquite. Ammonium thiocyanate also had no effect on 2,4,5-T activity on live oak (Quercus virginiana Mill.), huisache (Acacia farnesiana (L.) Willd.) and whitebrush (Aloysia lycioides Cham.). In addition, the activity of MCPA ester on whitebrush was not affected by ammonium thiocyanate and results with 2,4-D ester on Macartney rose (Rosa bracteata Wendl.) were inconsistent (Tschirley 1968). Thus ammonium thiocyanate has produced a large increase in 2,4,5-T activity in one instance but has otherwise generally

failed to produce any enhancement of activity, or the results have been inconclusive.

The activity of contact herbicides has also been increased by ammonium salts. For instance, control of hungerweed (Ranunculus arvensis L.) was increased from 0% to 88-100% kill when ammonium sulfate was added to sodium dinitro-o-cresylate (NaDNOC), with no injury from the salt applied alone (Harris 1940). Increased herbicidal activity has also resulted from the addition of ammonium sulfate to the sodium salt of pentachlorophenol (NaPCP) and the disodium salt of 3,6-endoxohexahydrophthalate (endothall) (Crafts and Reiber 1945; Tischler et al. 1951).

Potassium salts have also enhanced herbicide activity, commonly as much as ammonium salts. Russian thistle control by 2,4-D amine was increased by potassium phosphate from a rating of 1.0 to 4.0 (0-10 scale) (Sexsmith 1953). In this case, potassium phosphate was less effective than ammonium sulfate and ammonium phosphate, but equally effective as ammonium nitrate. Wills (1971, 1973) found that the potassium ion was equally effective as the ammonium ion in enhancing MSMA and dalapon activity on purple nutsedge while it was more effective than ammonium in increasing glyphosate activity. The effect of potassium chloride on 2,4-D activity is unclear. Makodzeba (1962) indicated that potassium chloride, besides ammonium nitrate or ammonium sulfate, could be added to 2,4-D Na salt to increase weed control in corn, but Zambin (1962) found that potassium chloride had no effect on 2,4-D

activity.

Sodium phosphate and sodium bisulfate are the only sodium salts which consistently increase herbicide activity. Increases in the activity of 2,4-D amine (Szabo and Buchholtz 1956), glyphosate (Wills 1973), MSMA and dalapon (McWhorter 1971) have resulted from adding sodium phosphates. McWhorter (1971) found that the toxicity of MSMA and dalapon was increased by the cations sodium, potassium and lithium, while the most effective anions were sulfate, phosphate, and nitrate. However, sulfate had no effect on 2,4-D amine activity (Sexsmith 1953) and sodium nitrate did not increase picloram activity (Turner and Loader 1972). Sodium bisulfate was equally effective as ammonium sulfate in enhancing the activity of NaDNOC and NaPCP (Crafts and Reiber 1945; Harris 1940).

Evidence on the adjuvant effect of calcium salts is sketchy. Chesalin (1962) and Ladonin (1961) found that superphosphate, which is mainly calcium monobasic phosphate, increased 2,4-D Na salt activity, but Zambin (1962) found that it had no effect. Also, calcium nitrate increased the toxicity of 2,4-D Na salt (Al'tergot and Kiselev 1963) but was antagonistic with picloram (Turner and Loader 1972).

Lithium salts appear to be effective adjuvants from the data presently available. Lithium was twice as effective as ammonium (both added as borate buffers) in increasing 2,4-D activity on bean epicotyl bending (Orgell and Weintraub 1957). In addition, lithium was as effective as sodium and potassium

ions on MSMA and dalapon activity on Johnson grass (Sorghum halepense (L.) Pers.) (McWhorter 1971). Magnesium borate was effective in increasing the activity of MCPA Na salt (Zambin 1962). Aluminum sulfate has only been investigated as an additive to NaDNOC and NaPCP, where it was as effective as ammonium sulfate (Crafts and Reiber 1945; Harris 1940).

Heavy metallic salts have had a marked antagonistic effect on herbicide activity. Salts of iron, copper, cobalt, and zinc reduced the activity of 2,4-D (Sexsmith 1953; Szabo and Buchholtz 1956; Wort 1962). The salts of iron and zinc also reduced glyphosate toxicity on purple nutsedge to that of the untreated control (Wills 1973).

In summary, it is evident that ammonium salts can produce very marked increases in the activity of herbicides, though not all ammonium salts increase activity. The salts which have been most effective are ammonium sulfate, ammonium nitrate, ammonium dibasic phosphate, ammonium monobasic phosphate, ammonium chloride and ammonium citrate. Ammonium thiocyanate, considered separately from other ammonium salts, is very effective with amitrole, but is inconsistent in affecting the activity of growth regulator-type herbicides in brush control. Other salts have not been investigated as thoroughly as ammonium salts, but potassium salts and certain sodium salts can be effective adjuvants. Lithium salts, magnesium borate and aluminum sulfate warrant further investigation. Heavy metallic salts consistently reduce herbicide activity.

Effects of Inorganic Salts on Herbicide Absorption and Translocation

Inorganic salts have increased herbicide absorption and translocation. The limited evidence available on the effect of ammonium salts pertains to ammonium nitrate and ammonium thiocyanate. Ammonium nitrate increased 2,4-D amine absorption by bean leaves from 46% to 76% of the amount applied and absorption by sunflower leaves from 15% to 56% (Szabo and Buchholtz 1961).

Absorption of 2,4,5-T isooctyl ester by four brush species was increased by ammonium nitrate (Brady 1970). The effect of ammonium nitrate ranged from causing a 1.77 fold increase in absorption by loblolly pine (Pinus taeda L.) to 3.81 fold increase in red maple (Acer rubrum L.). The amount of ^{14}C-2,4,5-T in the roots of post oak was increased threefold with ammonium nitrate added to the treatment solution compared with 2,4,5-T applied alone, but there was no increase in the other species despite the increase in absorption. This lack of an increase in translocation in three species, red maple, sweetgum (Liquidambar styraciflua L.) and loblolly pine, suggested the existence of a limiting step in the translocation mechanism not affected by the ammonium nitrate (Brady 1970).

Ammonium thiocyanate has increased translocation without an increase in absorption at the same time. The absorption of amitrole by Agropyron repens (L.) Beauv. was not increased by ammonium thiocyanate, but the amount translocated as a

percentage of the amount absorbed was increased from 14% to 29% (Donnally and Ries 1964). However, van der Zweep (1965) reported that ammonium thiocyanate did increase amitrole absorption by bean seedlings. Absorption of 2,4,5-T by cotton (Gossypium hirsutum L.) and winged elm was also not affected by ammonium thiocyanate, but translocation of 2,4,5-T was increased (Basler et al. 1967). For example, ammonium thiocyanate increased the amount of ^{14}C-2,4,5-T translocated from a treated leaf half way down the stem, to the lower half of the stem and attached leaves, from 3.7% to 18.8% of the amount applied.

There is little information available on the effect of other salts on herbicide absorption and translocation. Sodium dibasic phosphate was as effective as ammonium nitrate in increasing 2,4-D penetration through Sedum epidermis, and 2,4-D absorption by bean and sunflower leaves (Szabo and Buchholtz 1961). Phosphoric acid increased 2,4,5-T absorption by four brush species, with the magnitude of the increase as large as with ammonium nitrate on only two of the species (Brady 1970).

Heavy metal salts either had no effect or reduced 2,4-D absorption, with the exception of two salts. The absorption of 2,4-D by both beans and sunflower was increased by copper sulfate, while cobalt nitrate increased absorption by sunflower only (Szabo and Buchholtz 1961).

From the limited information available, it appears that inorganic salts may influence herbicide activity by an effect on herbicide absorption, and possibly translocation. However

only one attempt has previously been made to directly relate an increased activity effect to increased absorption or translocation, that is, the enhancement of amitrole activity by ammonium thiocyanate was related to an increase in translocation. Further study of absorption and translocation may reveal whether these factors account for all the increased activity caused by an inorganic salt adjuvant, or whether other factors are also involved in the mode of action on inorganic salts. Further study of heavy metal salts appears unwarranted.

Factors Influencing Adjuvant Activity of Inorganic Salts

Salt Concentration or Rate

A wide range of salt concentrations in the treatment solution, or rates of salt per unit area, has been reported necessary for optimum enhancement of herbicide activity. In many of the experiments reviewed, the solution application volume per unit area was not reported, so it is not possible to interchange concentration and rate per unit area. An attempt is made to review this subject with a consideration of both salt concentration in treatment solutions and amount of salt applied per unit area.

Concentrations of between 5% and 10% of ammonium sulfate and ammonium nitrate were reported as the best range for increasing the activity of 2,4-D Na salt (Al'tergot and Kiselev

1963; Borodna et al. 1962; Makodzeba 1962). Rates of 5 to 15 kg/ha of these salts were most frequently used in similar research with 2,4-D Na salt, (Chesalin 1962; Ladonin 1961; Likholat 1962) although Likholat (1962) found in subsequent trials that 3 to 5 kg/ha was sufficient. However the activity of 2,4-D can be increased at lower salt concentrations than reported above. The control of Russian thistle by 2,4-D amine was increased by ammonium sulfate at 2.4% and ammonium nitrate at 1.6%, but concentrations one-fourth these values, 0.6% and 0.4% respectively, were equally effective (Sexsmith 1953).

The response of picloram activity to a range of ammonium nitrate rates from 1.0 to 20 kg/ha was studied on guava, by measuring mean weight of new growth 35 days after treatment (Turner and Loader 1972). The rate of 2.5 kg/ha was sufficient for maximum effectiveness and higher rates did not cause any further significant decrease in regrowth. At the application volume of 148 to 352 L/ha, 2.5 kg/ha equals a concentration of 1.7% to 0.71%. In another experiment with four ammonium salts: nitrate, sulfate, chloride and citrate, there was commonly little difference between the effects of salt rates of 2.5 and 10 kg/ha. Thus a rate of 2.5 kg/ha of ammonium salts appears to be the lowest rate sufficient to produce maximum increase in picloram activity.

Turner and Loader (1972) also studied the effect of different ammonium nitrate concentrations on the activity of MCPA salt and ester formulations on willow and poplar. The lowest

ammonium nitrate rate of 5 kg/ha was as effective as the higher rates of 10 and 20 kg/ha.

In other research involving concentration effects, the ammonium enhancement of dalapon and MSMA activity was best at a salt concentration of 0.25% to 1.0% (Wills 1971). NaNAA activity on apple leaf curvature was increased by ammonium sulfate, ammonium nitrate and ammonium chloride at concentrations of 0.003% to 0.026%, with little difference in effectiveness between the concentrations (Horsfall and Moore 1962). Thus 0.003% is the lowest reported concentration at which inorganic salts have been successful adjuvants. Other salts have been effective at concentrations similar to those of ammonium salts (McWhorter 1971; Wills 1971, 1973; Sexsmith 1953).

Ammonium thiocyanate at rates of 1.12 to 4.48 kg/ha progressively increased the phytotoxicity of amitrole to Agropyron repens (L.) Beauv., but at 8.96 kg/ha ammonium thiocyanate had no effect on activity (Donnalley 1964). Hence there appears to be an optimum ammonium thiocyanate rate above which the adjuvant effect is reduced to zero. Much lower rates of 0.224 kg/ha (Elwell 1968) and 0.14 kg/ha (Hull 1967) increased the activity of 2,4,5-T on brush. The absorption of both amitrole and 2,4,5-T was enhanced by similar concentrations of ammonium thiocyanate. Ammonium thiocyanate increased amitrole translocation at 0.125% and 0.50% but had no effect at 2.0% (Donnalley 1964). The translocation of 2,4,5-T was increased at 0.25% and 0.50% ammonium thiocyanate but not at

lower concentrations of 0.02% to 0.10% (Basler et al. 1967). The failure of 2.0% ammonium thiocyanate to increase amitrole translocation indicates the existence of an optimum concentration above which the adjuvant effect decreases, as with the activity data.

Reports of the concentration or rate of salt necessary for maximum increase of NaDNOC activity vary widely. Harris (1940) tested a range of 1.12 - 11.2 kg/ha of ammonium sulfate added to NaDNOC, and found 5.60 to 8.96 kg/ha sufficient to give maximum effect in the control of nightshade (Solanum nigrum L.) in peas. A much higher ammonium sulfate rate of 67.2 kg/ha, from a tested range of 16.8 to 89.6 kg/ha, was the minimum rate necessary to add to NaDNOC to produce 100% kill of hungerweed (Harris 1940). Crafts and Reiber (1945) reported that only low concentrations of ammonium sulfate and sodium bisulfate were necessary to produce enhancement of NaDNOC and NaPCP. For instance they tested a concentration range of 0.025 to 0.400% of ammonium sulfate and sodium bisulfate and found that 0.025 to 0.100% was sufficient for maximum effectiveness of NaDNOC on Amsinckia douglasiana DC. and Brassica nigra (L.) Koch.

It is apparent that a salt concentration approximately within the range of 0.1% to 1.0% can result in a maximum enhancement of herbicide activity, absorption or translocation. A range of rates of 2.5 to 10 kg/ha appears to be adequate. However a much higher concentration on the order of 10%, or a rate possibly as high as 90 kg/ha, may be necessary. On

the other hand a concentration as low as 0.003% may be sufficient for maximum enhancement of activity of some herbicides. Thus in evaluating inorganic salts for adjuvant activity, a very wide range of concentrations is necessary for determining an adequate response curve.

Plant Species

Inherent plant characteristics are a factor in herbicide activity, absorption and translocation, consequently the adjuvant effect of inorganic salts may vary considerably between plant species. This was demonstrated in the absorption of 2,4,5-T ester by four brush species (Brady 1970); ammonium nitrate increased 2,4,5-T ester absorption in red maple, sweetgum, post oak, and loblolly pine by varying degrees. A marked adjuvant by species interaction was apparent in this experiment. For example ammonium nitrate increased 2,4,5-T ester absorption in red maple more than phosphoric acid did, but phosphoric acid was more effective in increasing absorption in post oak. In another study involving different species, ammonium sulfate increased control of Atriplex patula L. and Fumaria officinalis L. by NaDNOC, but did not effect NaDNOC activity on Polygonum convolvulus L. and Polygonum aviculare L. (Blackman et al. 1949). Basler et al. (1967) found that the effect of ammonium thiocyanate on the translocation of 2,4,5-T ester varied with the species studied, producing an increase in

translocation in cotton and winged elm seedlings but not in blackjack oak.

It is evident that the adjuvant effect of an inorganic salt can depend on the plant species involved. In seeking to increase the activity of a herbicide on a particular weed, it will be necessary to test a wide range of salts at a range of concentrations, to be sure of finding the best adjuvant. Parts of this review indicate the salts and concentrations most likely to be effective.

Herbicide Formulation

Herbicide formulation is an important factor determining the absorption and translocation characteristics of an herbicide (Norris and Freed 1966). Therefore an interaction of formulation and inorganic salt adjuvants might occur. However ammonium nitrate enhanced the activity of both the salt and ester form of MCPA (Turner and Loader 1972). On willow, for example, ammonium nitrate increased MCPA salt injury at 13 days from 80% to 92%, and increased MCPA ester injury from 65% to 84% although with the ester, the enhancement effect did not persist at 11 months after treatment. In contrast, Turner and Loader (1972) stated that ammonium nitrate increased the effects of the salt of 2-((4-chloro-o tolyl)oxy)propionic acid (mecoprop) on privet, but was antagonistic with mecoprop ester. Other reports have not directly compared the response of different formulations,

but inorganic salts have increased the activity of 2,4-D formulated as the Na salt (Al'tergot and Kiselev 1963; Borodna et al. 1962; Chesalin 1962), and both the activity and absorption of the triethanolamine salt (Szabo and Buchholtz 1956, 1961). The absorption of the isooctyl ester of 2,4,5-T was increased by ammonium nitrate, while ammonium thiocyanate has increased the activity and translocation of the butoxyethanol ester of 2,4,5-T, applied in both cases in oil-water emulsions (Basler et al. 1967; Elwell 1968).

It is apparent that inorganic salts can enhance the activity, absorption and/or translocation of herbicides in different formulations - inorganic salts, amine salts, and esters. With the information available at present, there is no reason to select one formulation over another, in seeking to increase the activity of an herbicide by the use of inorganic salts.

Mechanisms of Action of Inorganic Salt Adjuvants

Influence of pH

Foliar absorption of herbicides is influenced by the pH of the treatment solution (Hull 1970), so any variation in the pH of herbicide solutions caused by the addition of inorganic salts, must be considered as a factor in the mechanism of action of salt adjuvants. The absorption of herbicides which are weak acids such as 2,4-D, picloram, dalapon, and DNOC is typically inversely related to the pH

of the external solution (Crafts 1953; Sargent and Blackman 1962, 1970; Sargent et al. 1969; Simon 1953). Sargent and Blackman (1962) studied the absorption of 2,4-D from 0.15 ml of 2,4-D solution in potassium monobasic phosphate buffer, maintained in a plastic tube adhered to leaf discs of Phaseolus vulgaris L. As the pH was decreased from 6.4 to 4.0, absorption increased markedly; at pH values from 6.4 up to 9.4, absorption remained relatively constant. Using the same method, Sargent and Blackman (1970) found that picloram absorption from potassium biphthalate buffered solutions increased in a slightly inverse parabolic curve as pH was reduced from 7.2 to 4.2. Baur et al. (1971) measured picloram absorption by maintaining detached live oak leaves with the lower half dipped in the treatment solution. Absorption decreased significantly between pH 6 and 7, but there was no difference between pH 4 and 6, as might be expected.

Several experiments provide evidence on the role of pH in the effect of inorganic salt adjuvants on herbicide absorption. The effect on bean epicotyl bending, of 2,4-D applied in an ammonium phosphate buffered solution differed from the effect of 2,4-D applied in sodium (citrate, phosphate and borate) buffer solutions. At low pH values of two to three, activity was high in both cases and declined with decreasing hydrogen ion concentration to about pH 5.5. However while 2,4-D activity from sodium buffer solutions continued to decrease at more alkaline pH values, activity from

the ammonium buffer solution increased again to a maximum near pH 8, and then declined at still greater alkalinity. The epicotyl bending at pH 8 from the ammonium buffer solution reached a peak of 55° to 65° compared with a neglibible value for the Na buffer curve at pH 8. Thus the ammonium phosphate enhancement of 2,4-D activity around pH 8 is an effect separate from the increase in 2,4-D activity caused by reducing the pH of the treatment solution.

Further evidence comes from experiments where the herbicide alone and herbicide plus salt were maintained at the same pH, or where the salt did not produce a pH reduction but did produce an enhancement effect. Szabo and Buchholtz (1961) measured penetration of 2,4-D triethanolamine salt through Sedum epidermis by mounting the epidermis between two solutions with pH adjustment by hydrochloric acid or sodium hydroxide. Ammonium nitrate and sodium dibasic phosphate increased 2,4-D penetration at pH 5 from 9.3% to 13.5% and 12.7% respectively. Turner and Loader (1972) found the pH of picloram solutions to be 5.9 and 6.2 depending on picloram rate, and that adding ammonium nitrate, sulfate or chloride did not markedly change the pH. Hence the enhancement of picloram activity was probably not due to changes in the pH of the spray solutions.

The effect of ammonium sulfate on NaDNOC activity has been attributed to the pH reduction in the NaDNOC solution, resulting in increased absorption of DNOC (Crafts and Reiber 1945; Robbins et al. 1942). However Blackman et al. (1949)

and Simon (1953) did not agree with this explanation because NH_4DNOC has shown greater activity than DNOC. Also, the addition of 0.017% ammonium sulfate to NaDNOC solution has increased the control of Convolvulus arvensis L. from 11% to 62%, with a negligible change in pH from 7.43 to 7.34 (Crafts and Reiber 1945).

Hence it appears at least that ammonium salts can cause an increase in herbicide absorption that cannot be attributed to a pH effect. However a pH reduction may still be involved in an enhancement effect, so in determining the mechanism of action of inorganic salt adjuvants, any pH change caused by the salts must be investigated for its role influencing herbicide absorption.

Experiments involving inorganic salt adjuvants and pH effects, can involve procedures or results that do not permit a conclusive interpretation. Sargent and Blackman (1962) reported that if pH values in phosphate buffered solutions are initially low, the pH increases when the solution is maintained on the leaf surface in an absorption experiment. Szabo and Buchholtz (1961) attempted to measure the effect of ammonium nitrate and sodium dibasic phosphate on 2,4-D amine absorption by bean and sunflower leaves with the treatment solutions adjusted to pH 5 or pH 3. The pH of the solutions was adjusted with hydrochloric acid or sodium hydroxide and each treatment solution was applied as a 0.1 ml droplet to the leaf. Where a small volume of treatment solution is added to a leaf, and the solution does not

contain a buffer intended to maintain the desired pH, then during the absorption period, the pH could shift even more than in the experiment of Sargent and Blackman (1962). In attempting to elucidate the role of pH in a salt enhancement effect, there must be control of, or a knowledge of the pH during the course of the experiment.

Effect of Inorganic Salts on Stomatal Penetration

Stomatal penetration by applied substances may result in increased absorption, but the stomata are not direct portals of entry to the leaf (Robertson and Kirkwood 1969). Substances that enter the substomatal cavity must penetrate the so-called internal cuticle, although this is normally thinner and may differ in composition to the external cuticle (Crafts and Foy 1962; Goodman and Addy 1963). However aqueous solutions will not penetrate stomata unless the surface tension of the solution is reduced (Currier and Dybing 1959; Dybing and Currier 1959, 1961). Thus if inorganic salts reduced surface tension this could result in stomatal penetration by the treatment solution and possibly an increase in absorption. However surface tension values for aqueous salt solutions in Lange's Handbook of Chemistry (1967) reveal that inorganic salts cause small increases in surface tension, generally less than 1.0 dyne/cm at 0.5M salt concentration. Furthermore, salts added to 2,4-D solution resulted in only very small changes in surface tension which did not correlate

with penetration effects (Szabo and Buchholtz 1961).

Cuticular Penetration

In order to consider the possible modifying effect of inorganic salts on cuticular penetration, it is appropriate to first review the nature and composition of cuticle. A cuticularized epidermal cell wall is composed of four substances - cutin, cutin waxes, pectin and cellulose (Frey-Wyssling 1948). The cuticle proper is the outermost layer of only wax and cutin (Crafts and Foy 1962; Sitte and Rennier 1963). Between the cuticle and cellulose cell wall there is a layer of pectin. Wax is also present on the cuticle surface as usually irregular deposits, known as surface or epicuticular wax (Crafts and Foy 1962; Hull 1970).

The wax within the cuticle may be present as scattered lamellae as diagrammed by Orgell (1954), or it may be a continuous layer (Norris and Bukovac 1968). It is hydrophobic and thus a major barrier to penetration of polar aqueous solutions (Foy et al. 1967). Cutin constitutes the matrix of the cuticle and evidence indicates it is also a major barrier to penetration of polar compounds (Crafts and Foy 1962; Darlington and Barry 1965). Cutin is a polyester of long-chain fatty acids including hydroxylated and both mono- and dicarboxylic acids, and long chain alcohols (Hull 1970; Matic 1956; Roelofsen and Houwink 1951). It contains both polar hydrophilic groups (-OH and -COOH) and nonpolar

lipophilic groups ($-CH_2-$ and $-CH_3$) (Franke 1967). It is thus semipolar in nature. Pectin consists of long-chain polygalacturonic acid molecules with side carboxyl groups which impart base exchange properties (Foy et al. 1967). Both pectin and cellulose are polar and thus not important obstacles to the penetration of substances in aqueous solution (Crafts 1961; Foy et al. 1967; Franke 1967).

Polar (aqueous) and nonpolar (lipoidal) routes of penetration through the cuticle have been proposed (Crafts 1961; Foy 1964). Under this proposal, substances in aqueous solution, such as inorganic salts, acids, bases and polar compounds, may enter through cracks, punctures, or areas of leaves not completely covered by waxy lamellae, and then follow the polar route. In contrast, lipoidal compounds penetrate by the nonpolar route by solubilizing directly into the waxy portions of the cuticle (Crafts 1961; Foy 1964; Foy et al. 1967). For substances exhibiting both polar and apolar pathways, it is envisaged that entry likely occurs via a combined pathway (Foy 1964).

The sorption (adsorption plus absorption) of acidic substances by isolated apricot cuticle responds in a somewhat similar manner to pH of the external solution as does the foliar absorption of weakly acidic herbicides (Orgell 1954). As reviewed above absorption is high at low pH values and is reduced as pH is increased, so that absorption is relatively low from alkaline solutions (Crafts 1953; Sargent and Blackman 1962, 1970; Sargent et al. 1969; Simon 1953). The proposed

explanation for this response curve is in terms of cuticle charge and degree of dissociation of the applied molecules. Cutin has a negative charge at neutral or high pH due to dissociation of the polar groups (Crafts and Foy 1962; Franke 1967; Orgell 1957). The applied weak acid will be dissociated at neutral or high pH, so that electrostatic repulsion could slow foliar absorption or sorption of the anion. At low pH, the acid substances are undissociated, relatively nonpolar, and hence readily soluble in the cuticle. Absorption by solubilizing in the cuticle constitutes the proposed lipoidal route of absorption (Crafts 1961; Foy 1964; Foy et al. 1967).

Increased absorption at low pH has also been attributed to reduced ionization of the polar groups in the cuticle (Crafts 1961). The absorption of picloram has been increased by reducing solution pH from pH 7 to 6, a change which has neglible effect on decreasing picloram dissociation (Baur et al. 1971; Sargent and Blackman 1970). Therefore the pH effect, at least at this pH range, appears to be largely on the plant. The acidic groups of the cutin have a pK value above five, which is higher than picloram (pKa 3.1, Sargent and Blackman 1970) so the pH reduction may suppress the negative cuticular charge and so increase picloram anion absorption (van Overbeek 1956). This proposal of van Overbeek (1956) related to a similar relationship between pH and activity of 2-methyl-4-chlorophenoxyacetic acid. Reducing the solution pH has also resulted in an increased activity of

2,4-D on bean epicotyl bending which is not related to decreased 2,4-D dissociation (Orgell and Weintraub 1957).

It is possible that inorganic salts may act in a similar manner as hydrogen ions to increase cuticular penetration of herbicide anions, by reducing the cuticular negative charge. If high hydrogen ion concentration and inorganic salts act to increase absorption by a common mechanism, then absorption of herbicides enhanced by inorganic salts should not exceed the absorption which occurs at low pH. The results of Orgell and Weintraub (1957) conform to this. Briefly, 2,4-D applied to beans in an ammonium buffer at pH 8 caused epicotyl bending of 55° to 65° compared with a value of 5° for the sodium buffer, but below pH 5.5, epicotyl bending was not affected by the buffer in which the 2,4-D was applied. Ammonium nitrate has increased 2,4-D penetration through Sedum epidermis at pH 3 from 16.1% to 23.5% of the amount added (Szabo and Buchholtz 1961). However penetration was measured from treatment solutions with pH adjusted with hydrochloric acid, through the epidermis, to distilled water. The pH of the solution may not have been maintained at a low value if hydrogen ions readily penetrated through to the distilled water. The suggestion that salt enhanced absorption should not exceed absorption at low pH is not contradicted, as the validity of the above experiment is questionable, but the only supportive evidence is 2,4-D activity data.

If inorganic salts act by reducing the cuticular negative charge, then cations must be primarily responsible for

the enhancement effect. Ion binding by isolated cuticle from tomato fruit and onion leaf has been studied by floating cuticle on treatment solutions for 5 seconds. Binding of the cations calcium and rubidium was much greater than binding of sulfate and chloride anions (Yamada et al. 1964a). In another study of binding over a 5 minute period, the total binding capacity for the calcium ion was up to 500-fold that for the sulfate ion (Yamada et al. 1964b). The fixation of calcium reaches an equilibrium in 3 minutes whereas sulfate required 200 minutes, and exchange removed all the sulfate, but 26 to 44% of the calcium was retained. These results indicate that cuticle does have a much stronger affinity for cations than anions.

Herbicides penetrating the cuticle via the proposed lipoidal route are directly soluble in the cuticle and would not be affected by cuticular charge (Crafts 1961; Foy 1964; Foy et al. 1967). Hence inorganic salts could not increase herbicidal penetration via the lipoidal route by the mechanism proposed above. However at present the enhancement of absorption of herbicides penetrating the cuticle via this proposed route has not been reported. The isooctyl ester of 2,4,5-T is lipid soluble, but when ammonium nitrate increased the absorption of this 2,4,5-T ester, pH of the solution was 4, so it was concluded that some of 2,4,5-T probably was hydrolyzed (Brady 1970). The hydrolyzed portion would be absorbed via the aqueous route and hence its absorption could be enhanced by ammonium nitrate by the proposed mechanism.

Action of Inorganic Salts on Herbicide Translocation

Ammonium thiocyanate has been reported to directly affect herbicide translocation. Forde (1966) studied the translocation patterns of amitrole and ammonium thiocyanate in quackgrass and concluded that the effect of ammonium thiocyanate is exerted in the treated leaf rather than at the site of action of amitrole. Ammonium thiocyanate stopped movement of amitrole from the treated leaf in the first 12 hours, but by 24 hours, movement of amitrole was increased by the added ammonium thiocyanate. The conclusion was based on this initial block of translocation and the lack of movement of ammonium thiocyanate from the treated leaf.

This effect in the treated leaf can possibly be explained by the reduced formation of the primary conjugate of amitrole, 3-(3-amino-1,2,4-triazolyl)-2-propionic acid, when ammonium thiocyanate is added (Carter 1965, 1967). As a result, amitrole in the leaf was increased from 66% to 97% of the ^{14}C present. In reporting that ammonium thiocyanate enhanced translocation of amitrole, Donnalley and Reis (1964) and Forde (1966) did not check the translocated ^{14}C for the presence of this conjugate. However, if the conjugate is less mobile than amitrole, then the increased availability of amitrole could account for the increased translocation of ^{14}C.

CHAPTER III

AMMONIUM SULFATE ENHANCEMENT OF PICLORAM HERBICIDAL ACTIVITY AND ABSORPTION IN TWO GUAVA SPECIES AND DWARF BEANS

Guava (Psidium guajava L.) is a serious weed in tropical areas such as Hawaii, Puerto Rico, and Fiji (Cates 1967; Motooka et al. 1967a; Tschirley et al. 1967). Strawberry guava (Psidium cattleianum Sabine) presents a similar serious problem (Plucknett 1969). Control of guava by conventional mechanical methods is both uneconomical, and in many areas physically non-feasible, so that an effective and economical method of chemical control is needed. The most effective herbicide for long term defoliation of guava is the potassium salt of 4-amino-3,5,6-trichloropicolinic acid (picloram) (Tschirley 1968), but its effectiveness is still less than desired (Bovey et al. 1969; Tschirley 1968; Tschirley et al. 1967).

The objective of this study was to increase control of guava and strawberry guava by using an inorganic salt to increase picloram herbicidal activity. Ammonium sulfate was chosen from preliminary experiments, and in this study the effects of picloram rate and salt concentration on the enhancement of picloram activity were examined. Dwarf beans (Phaseolus vulgaris L. cv. Bountiful) were used as an additional test species in elucidating the effect of ammonium sulfate on picloram activity.

Only one attempt has previously been made to determine the mode of action of an inorganic salt that has successfully increased herbicidal activity. In this instance ammonium thiocyanate increased the activity of amitrole and the effect was related to increased amitrole translocation (Donnalley 1964). Another objective of this study was to examine the effect of ammonium sulfate on the absorption and translocation of picloram, as a possible means of explaining the enhancement of its activity.

Materials and Methods

Effect of ammonium sulfate on picloram herbicidal activity on guava and strawberry guava seedlings: Seedlings of guava and strawberry guava were grown from the seeds of fruits collected from trees at the Lyon Arboretum, Honolulu. The seeds were germinated in vermiculite, and 2 to 3 weeks after emergence were transplanted into pots 10 cm x 17 cm tall, containing one part of perlite to three parts of heat sterilized soil. The plants were kept outdoors for 3 to 4 months for guava, and 4 to 5 months for slower-growing strawberry guava. Approximately 0.4 g of a complete fertilizer of 10:10:10 was added to the pots every 3 to 4 weeks. The seedlings were transferred to the glasshouse, where the temperature was several degrees higher than outside. Conditions in the glasshouse were summer: 30 ± 5 C, 57 ± 20% RH day, 21 ± 1 C, 90 ± 10% RH night, and winter: 25 ± 5 C, 65 ± 20% RH day, 18 ± 1 C, 90 ± 10% RH night. The seedlings

were treated 2 months later, after a flush of growth. The guavas were single stemmed 50 to 60 cm tall; the strawberry guavas were multi-stemmed and 30 to 40 cm tall. Six plants per treatment were selected, and the experiment on each guava species was repeated once.

The seedlings were sprayed with a hand carried, compressed nitrogen sprayer calibrated to deliver 140 L/ha at 1.4 kg/cm^2. Picloram K salt (Tordon 22K[1]) rates of 0.25, 0.50, and 0.75 kg a.i./ha were applied in tap water, with ammonium sulfate added at concentrations of 0.10, 0.75, 1.0, and 10.0% (w/v). The top of each pot was filled with perlite to stop any picloram action via the soil. The perlite was removed soon after spraying. The plants were returned to the glasshouse, and thereafter the leaves were not wetted during watering.

Results were recorded as an injury rating on a 0-10 scale; 0 equals no injury, to 10 equals all leaves dead. The rating was based on the percentage of the leaves that were either necrotic (dessicated) or had fallen from the plant, plus an adjustment for the extent of chlorosis and necrosis of the remaining leaves. The fresh weight of live leaves was measured when the experiment was terminated.

Effect of ammonium sulfate on picloram herbicidal activity on dwarf bean seedlings: Dwarf bean seedlings

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[1]Trade name of Dow Chemical Company, Michigan.

were grown in pots, 8 cm x 11 cm tall containing silica sand, in the glasshouse. The conditions were 31 ± 5 C, 50 ± 20% RH day and 23 ± 1 C, 90 ± 10% RH night. After emergence, the beans were watered with one-half strength nutrient solution modified from Hoagland and Arnon (1938). The leaves were not wetted with the nutrient solution. The plants were sprayed at 9 to 10 days after planting when the first trifoliate leaf was beginning to unfold. The treatment procedure was the same as for the guava plants, but with cardboard across the top of each pot to prevent picloram contamination of sand. Picloram K salt (Tordon 22K) rates of 0.02 and 0.04 kg a.i./ha, and ammonium sulfate concentrations of 0.1, 0.5, 1.0, 5.0, and 10% (w/v), were applied in deionized water to six plants per treatment. The experiment was repeated once. Response of the bean seedlings was assessed weekly on a 0 to 5 scale; 0 equals no effect, 5 equals plants dead. A rating of 1, 2, or 3 was based largely on petiolar and epicotyl bending; chlorosis was an additional factor in a rating of 4.

Effect of ammonium sulfate on absorption and translocation of picloram by the guava species: Carboxyl-labelled ^{14}C-picloram (specific activity 4.13 μCi/mg) was converted to the potassium salt using commercial formulation blank[1], so that the ^{14}C solution was equivalent to Tordon 22K. The recommended proportion was 21.5 : 78.5 (w/w) picloram acid

[1] Supplied by Dow Chemical Company, Michigan.

to formulation blank. A slight excess of formulation blank was used and amounted to 0.1% (w/v) of the final treatment solution. The ^{14}C-picloram K salt (hereinafter referred to as ^{14}C-picloram) was diluted with technical grade picloram K salt (76.8% a.i.) to a final total picloram concentration of 1000 ppmw a.i. Ammonium sulfate was added to the appropriate treatment solutions. The effect of ammonium sulfate on picloram absorption and translocation was investigated at 0.5% and 10% (w/v) ammonium sulfate on strawberry guava, and 0.5% on guava. Three plants per treatment were used, and each experiment was repeated once. The first mature pair of leaves on the upper stem of each guava or strawberry guava plant, was treated with two 5 μL droplets per leaf (0.05 μCi per plant). The droplets were placed on the upper leaf surface, one on each side of the midrib and between the main lateral veins. Two days later, the treated leaves were washed with 50 ml of distilled water, and the plants freeze-dried. Only that portion of each plant, from above the node below the treated leaves, was retained for measurement of the ^{14}C-label. For quantitative determination of the ^{14}C, this portion of each plant was separated into a) treated leaves and b) remainder, consisting of upper stem and attached leaves. The amount of ^{14}C in portion b) was taken as the amount of ^{14}C translocated. The samples were ground with a Wiley mill, oxidized, and the $^{14}CO_2$ trapped in a liquid scintillation cocktail, using the apparatus and methods described by Peterson (1969) and Peterson

et al. (1969). The samples were counted in a Packard liquid scintillation spectrophotometer.

The effect of 0.5% (w/v) $(NH_4)_2SO_4$ on picloram absorption and translocation was also examined using autoradiography. The apical portion of each of two plants per treatment was mounted and exposed to No-Screen X-ray film for 3 months, according to the methods of Crafts and Yamaguchi (1964).

Effect of ammonium sulfate on absorption of picloram by dwarf bean: The beans were grown in the glasshouse as described, and transferred to a growth chamber one day before treatment. Conditions in the chamber were 30 C day, 24 C night, 50 ± 2% RH, light intensity of 23500 lux, and a photoperiod of 13 hours. Primary leaves were treated with 50 L placed as 16 to 18 droplets per leaf, or 1000 ppmw picloram K salt (Tordon 22K) alone, and plus 0.5% or 10% (w/v) ammonium sulfate. Absorption of picloram was measured by the difference between the amount applied to the leaf and the amount recovered from the leaf surface. After 24 hours each leaf was washed four times with 5 ml of distilled water. Additional washes did not remove any more picloram. Furthermore, the amount of picloram applied could be fully recovered immediately after application. Picloram concentration in each wash was measured by the method of Cheng (1969). One ml of concentrated sulfuric acid and 1 ml of 0.1 M sodium nitrite was added to each 5 ml sample. After

10 minutes for color development under yellow light, the concentration of picloram was read directly from a calibrated Unicam spectrophotometer at 405 nm. The amount of picloram applied was checked with a spectrophotometer reading, and the percentage absorption calculated by the difference.

Picloram metabolism: The picloram extraction procedure of Merkle and Davis (1966) was used. The tissue was homogenized for 2 minutes in acidified acetone (4 ml concentrated hydrochloric acid per L) in an approximate 5:1 solvent: tissue ratio, filtered, washed with acidified acetone, and the extract evaporated to dryness in vacuo. The residue was taken up in 25 ml of 0.1N potassium hydroxide solution, adjusted to pH 8.5, and washed with four equi-volumes of diethyl ether. The alkaline extract was further adjusted to pH 2 with hydrochloric acid, and the ^{14}C-label extracted with four equi-volumes of diethyl ether. The ^{14}C in diethyl ether was chromatographed used descending paper chromatography and a solvent system of benzene : propionic acid : water (2:2:1 v/v), based on the method of Redeman et al. (1968). A ^{14}C-picloram acid standard for cochromatography was prepared by adding ^{14}C-picloram acid to filtered extract from untreated tissue, and following the remaining extraction procedures.

The ^{14}C on the paper chromatographs was counted by first cutting each chromatograph into 11 strips. Each strip,

representing one Rf value, was placed in a scintillation vial with 15 ml of a dioxane/naphthalene cocktail (Bray 1960), and counted in a Packard liquid scintillation spectrometer.

Results

Effect of ammonium sulfate on picloram activity on guava and strawberry guava: Ammonium sulfate increased the herbicidal activity of picloram on guava to varying degrees depending on the picloram rate and ammonium sulfate concentration (Table 1). Picloram injury to guava was enhanced by ammonium sulfate at all three picloram rates at 4 to 6 weeks after treatment. Picloram alone at the higher rates of 0.50 and 0.75 kg/ha continued to increase injury with time. By 8 weeks, the enhancement effect remained evident only at the low picloram rate, 0.25 kg/ha, where picloram plus ammonium sulfate was as effective as 0.75 kg/ha of picloram alone. Ammonium sulfate concentrations of 0.75%, 1.0% and 10% were equally effective at increasing picloram activity at 8 weeks, but at the earlier times, there was a tendency for picloram activity to increase with increasing ammonium sulfate concentration up to 10%.

Picloram was less active on strawberry guava (Table 2) than on guava. Ammonium sulfate enhancement of picloram activity varied with picloram rate and salt concentration in a similar manner as on guava.

Results of a repeat experiment for each guava species showed a similar effect of ammonium sulfate on picloram

TABLE 1. -- Effect of $(NH_4)_2SO_4$ added to foliar spray applications of picloram on guava seedlings.

Picloram rate	$(NH_4)_2SO_4$ concn	pH of solution	Injury rating[x] Time (weeks) 4	6	8	Fresh wt. of live leaves 8
(kg/ha)	(%)					(g/plant)
Control			0	0	0	7.3 a[y]
	0	8.1	1	1	2	6.3 ab
	0.10	7.4	1	3	5	4.7 bc
0.25	0.75	6.9	1	6	9	2.3 def
	1.0	6.8	1	4	8	3.3 cd
	10.0	6.3	2	7	10	0.5 fg
	0		1	5	8	2.8 de
	0.10		3	6	9	1.2 efg
0.50	0.75		3	7	9	1.3 efg
	1.0		4	9	10	0.2 g
	10.0		7	9	10	0 g
	0	8.2	4	7	9	1.2 efg
	0.10	7.5	3	7	9	0.9 efg
0.75	0.75	7.0	5	9	10	0.2 g
	1.0	6.9	7	10	10	0 g
	10.0	6.4	8	9	10	0 g

[x]Scale 0 = no injury, 10 = all leaves dead; rating accounted for defoliation, necrosis and chlorosis.

[y]Means with the same letter are not significantly different at P = 0.05 (Duncan's Multiple Range test).

TABLE 2. -- Effect of $(NH_4)_2SO_4$ added to foliar spray applications of picloram on strawberry guava seedlings.

Picloram rate	$(NH_4)_2SO_4$ concn	Injury rating[x]				Fresh wt. of live leaves
		Time (weeks)				
		4	14	20	24	24
(kg/ha)	(%)					(g/plant)
Control		0	0	0	0	12.6 a[y]
	0	0	0.5	0.5	0.5	10.2 ab
	0.10	1	2	3	3	7.2 bcde
0.25	0.75	1	2	3	2	7.9 bcd
	1.0	1	2	3	3	5.8 cde
	10.0	2	4	5	4	4.2 cedf
	0	1	1	2	2	8.3 bc
	0.10	1	3	3	3	5.9 cde
0.50	0.75	1	5	6	6	3.5 ef
	1.0	2	4	5	5	5.3 cde
	10.0	2	4	6	6	3.3 ef
	0	1	4	4	5	4.3 cdef
	0.10	1	3	4	5	6.0 cde
0.75	0.75	1	4	4	4	5.0 cdef
	1.0	2	4	5	5	3.9 def
	10.0	4	7	9	8	1.1 f

[x]Scale 0 = no injury, 10 = all leaves dead; rating accounted for defoliation, necrosis and chlorosis.

[y]Means with the same letter are not significantly different at P = 0.05 (Duncan's Multiple Range test).

activity, as the results presented (Table 1 and 2).

On both guava species, the injury symptoms were similar in all treatments - epinasty, followed by chlorosis, necrosis and defoliation. There was a greater tendency for defoliation to occur on strawberry guava than on guava seedlings. None of the treatments caused contact or rapid necrosis.

Effect of $(NH_4)_2SO_4$ on picloram activity on dwarf beans: The effect of ammonium sulfate on enhancement of picloram activity was more clear-cut with beans than with the two guava species (Figure 1 and 2). The lowest ammonium sulfate concentration to enhance picloram activity was 0.5% at 0.02 kg/ha picloram, and 0.1% at 0.04 kg/ha. Many of the differences between treatments became narrower with time. After 3 to 4 weeks, the lowest ammonium sulfate concentration to produce maximum enhancement was 1.0% with both picloram rates. Results of a repeat experiment were similar.

None of the treatments caused any contact necrosis on the beans. Treatment with ammonium sulfate alone at 0.5% and 10% did not injure or enhance the growth of the bean plants. The pH values of the spray solutions ranged from 7.6 for picloram alone, down to 5.6 with 10% ammonium sulfate added. These values are lower than those of the solutions applied to the guava species (Table 1), as deionized water was used in place of tap water.

FIGURE 1. -- $(NH_4)_2SO_4$ applied in picloram spray solution increased epinasty, as an index of herbicidal activity on dwarf bean seedlings 5 days after treatment.

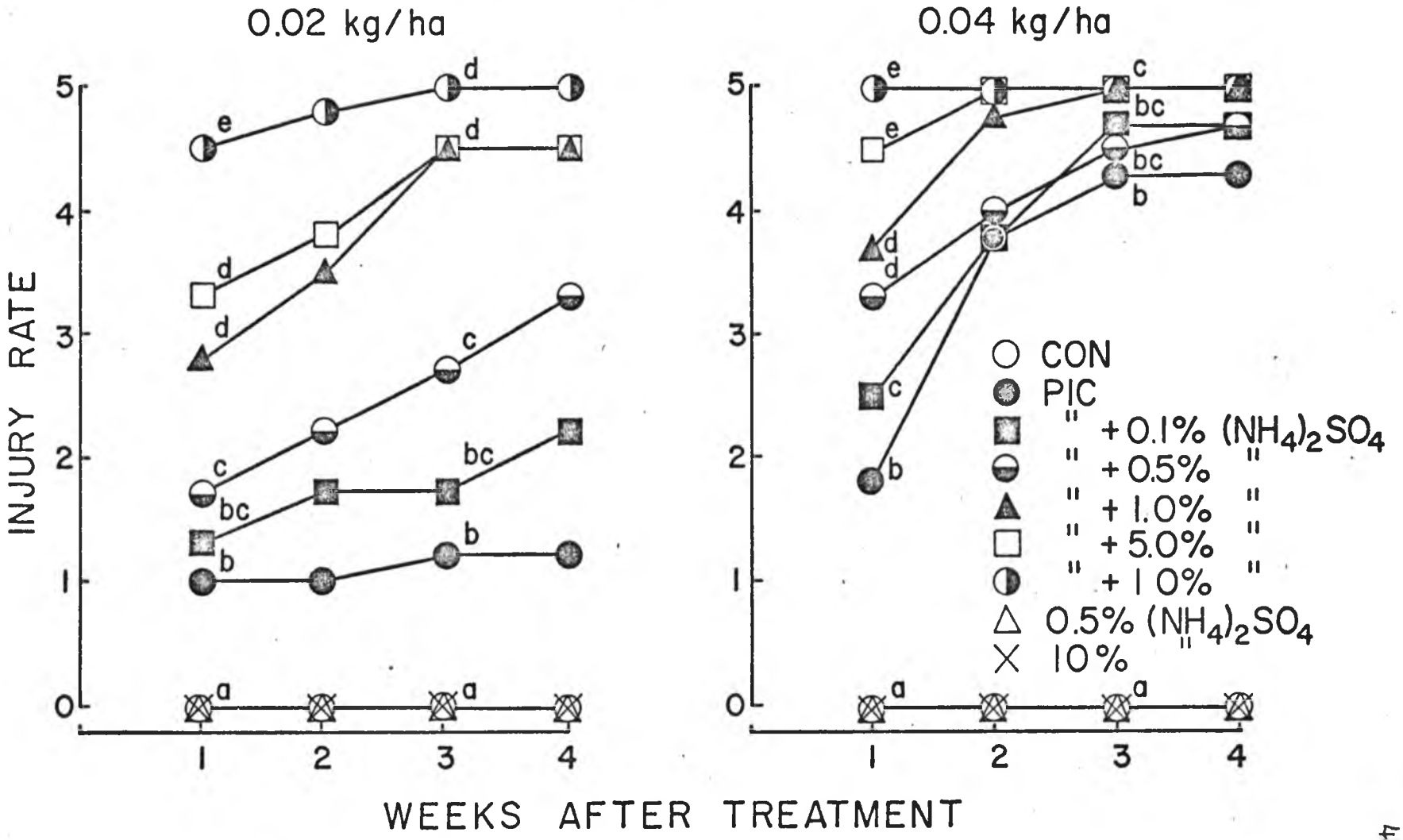
CONTROL PICLORAM 0.02 kg/ha

$(NH_4)_2SO_4$% 0 0.1 0.5 1.0 5.0 10.0

CONTROL PICLORAM 0.04 kg/ha

$(NH_4)_2SO_4$% 0 0.1 0.5 1.0 5.0 10.0

FIGURE 2. -- Effect of $(NH_4)_2SO_4$ applied in the spray solution on picloram activity on dwarf bean seedlings. The injury rating is on a scale 0 = no injury to 5 = plants dead. Means within an observation period at one picloram rate with the same letter are not significantly different at P = 0.05 (Duncan's Multiple Range test).


0.02 kg/ha
0.04 kg/ha
INJURY RATE
WEEKS AFTER TREATMENT
CON
PIC
" +0.1% (NH4)2SO4
" +0.5% "
" +1.0% "
" +5.0% "
" +10% "
0.5% (NH4)2SO4
10%

Effect of ammonium sulfate on absorption and translocation of picloram: The addition of 0.5% ammonium sulfate to the picloram solution increased total absorption of the ^{14}C by more than five-fold in strawberry guava, from 136 cpm to 720 cpm. The amount of ^{14}C translocated to the upper stem and attached leaves was increased by about four and one half-fold in the plants treated with 0.5% ammonium sulfate, compared with picloram alone (Table 3). Ammonium sulfate added at 10% did not result in an increase in absorption or translocation any more than did 0.5%.

Chromatography of extracts of translocated ^{14}C in a separate experiment indicated that all the ^{14}C was picloram (Table 4). Ammonium sulfate did not affect picloram metabolism by strawberry guava. Also all the ^{14}C in the treated leaves was picloram.

Autoradiographs show that the pattern of distribution of translocated ^{14}C was not changed by 0.5% ammonium sulfate (Figure 3). The leaves on the stem tip and the two small lateral branches were young and rapidly growing. The upper leaves were all labelled indicating picloram translocation to a site of high anabolic activity. In replicate strawberry guava plants from the same experiment, absorption of ^{14}C-picloram was increased from 510 cpm to 2238 cpm, and the amount translocated from 230 cpm to 768 cpm by 0.5% ammonium sulfate.

On guava plants, 0.5% ammonium sulfate increased ^{14}C absorption from 353 cpm to 1301 cpm. However this increase was largely accounted for in the treated leaves (Table 5),

TABLE 3. -- Effect of $(NH_4)_2SO_4$ on the absorption and translocation of ^{14}C-picloram after application of 0.025 μCi in two 5 μL droplets to each of one pair of leaves on strawberry guava seedlings for 2 days.

Treatment	pH of solution	^{14}C-Picloram in treated leaves		^{14}C-Picloram translocated	
		(cpm)	(S.D.)	(cpm)	(S.D.)
Picloram	10.0	78	10	58	27
Picloram + 0.5% $(NH_4)_2SO_4$	7.4	460	111	260	94
Picloram + 10% $(NH_4)_2SO_4$	6.4	506	273	264	136

TABLE 4. -- Paper chromatography of extracts of the translocated ^{14}C in strawberry guava and guava, and a ^{14}C-picloram standard. Chromatograph was developed in benzene: propionic acid:H_2O (2:2:1 v/v).

Treatment	Rf										
	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0
	(cpm)										
^{14}C-Picloram	0	0	0	0	21	480	54	0	0	0	0
Strawberry guava											
Picloram	0	0	0	0	3	46	4	0	0	0	0
Picloram + 0.5% $(NH_4)_2SO_4$	0	0	0	0	9	69	4	0	0	0	0
Picloram + 10% $(NH_4)_2S)_4$	0	0	0	4	10	61	0	2	0	0	0
Guava											
Picloram	0	0	0	0	4	114	86	2	0	0	0
Picloram + 0.5% $(NH_4)_2SO_4$	0	4	4	4	36	727	310	5	0	0	0

FIGURE 3. -- Distribution of ^{14}C-picloram in strawberry guava treated on one pair of leaves with 0.05μCi of ^{14}C-picloram in two 5μL droplets per leaf, alone (left) and plus 0.5% $(NH_4)_2SO_4$ (right). Top, plant portion; bottom, radioautograph.

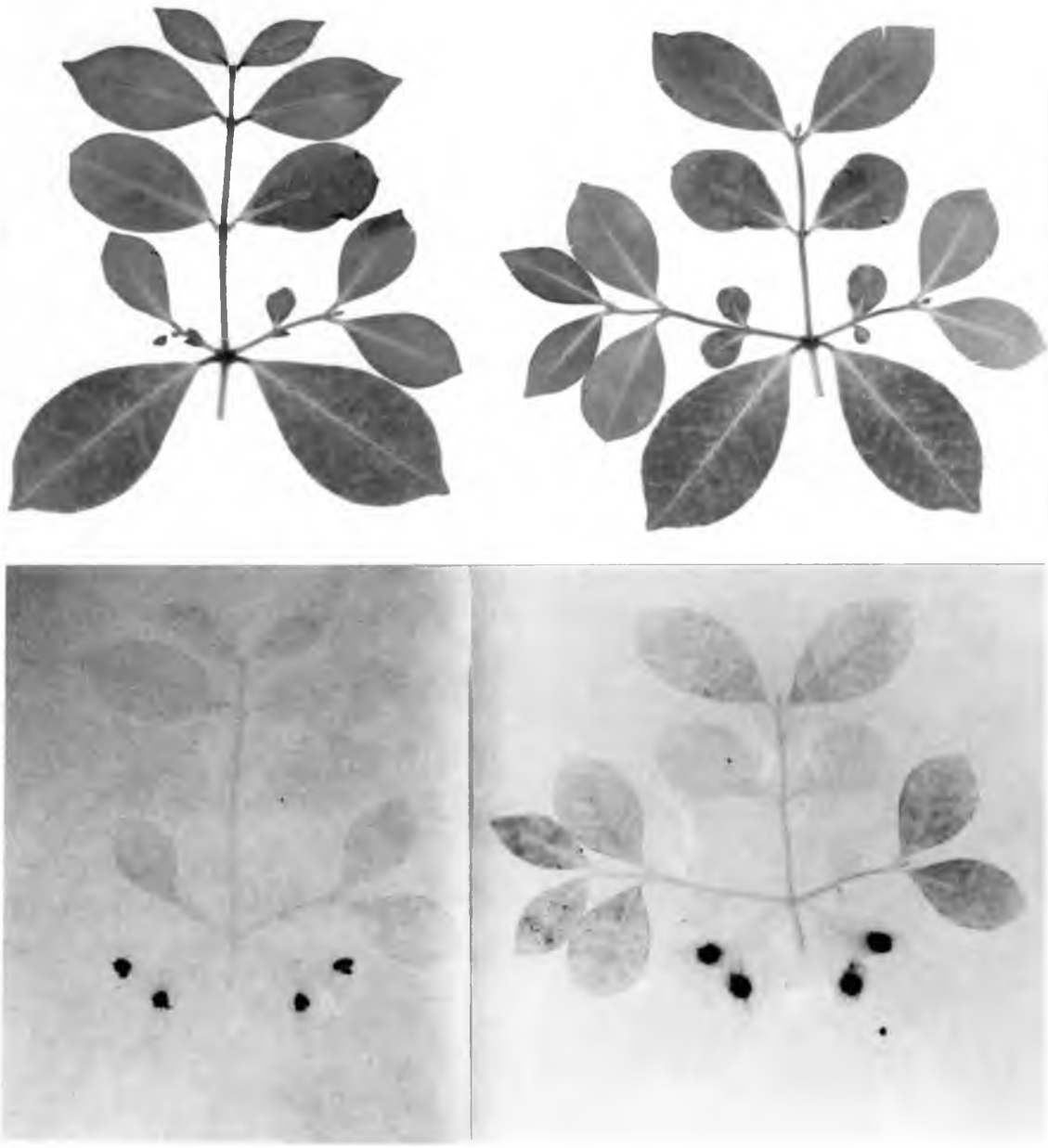


TABLE 5. -- Effect of $(NH_4)_2SO_4$ on absorption and translocation of ^{14}C-picloram after application of 0.025 μCi of ^{14}C-picloram in two 5 μL droplets to each of one pair of leaves on guava seedlings for 2 days.

Treatment	^{14}C-Picloram in treated leaves		^{14}C-Picloram translocated	
	(cpm)	(S.D.)	(cpm)	(S.D.)
Picloram	178	54	175	97
Picloram + 0.5% $(NH_4)_2SO_4$	1008	322	293	156

as the amount of ^{14}C which moved out of the treated leaves was not increased. There was no metabolism of ^{14}C-picloram in guava (Table 4).

Ammonium sulfate at 0.5% and 10% increased picloram absorption in beans by a similar magnitude as on guava species (Table 6). When treatment droplets containing ammonium sulfate occasionally produced necrosis on bean leaves, the plants were rejected.

TABLE 6. -- Effect of $(NH_4)_2SO_4$ on picloram absorption expressed as a percentage of the dose of 5 μg in 50 μL applied to each unifoliate leaf of dwarf bean seedlings. Plants were grown in the glasshouse and after treatment kept in the growth chamber for 1 day.

Treatment	pH of solution	Picloram absorption	
		(%)	(S.D.)
Picloram	7.8	1.8	0.8
Picloram + 0.5% $(NH_4)_2SO_4$	6.2	7.9	0.6
Picloram + 10% $(NH_4)_2SO_4$	5.8	5.6	1.1

Discussion

The ammonium sulfate enhancement of picloram activity on guava and dwarf beans agrees with the recent results of Turner and Loader (1972). My results also show that ammonium sulfate markedly increases picloram activity on strawberry guava.

Where a herbicide alone can cause a high degree of control at a sufficiently high rate, the potential of an adjuvant is to permit the same degree of control to be attained with a much lower rate. Thus when evaluating the effect of any adjuvant the herbicide rate must be sufficiently low to allow for maximum expression of the adjuvant effect. This was shown by the ammonium sulfate enhancement effect at different picloram rates on the two guava species and dwarf beans (Table 1 and 2, Figure 1 and 2). Ammonium sulfate caused a large increase in picloram activity at the low picloram rate in each experiment, whereas at the high picloram rate, the enhancement was only minimally evident, except relatively soon after treatment.

Ammonium sulfate at 1% was sufficient to produce maximum enhancement of picloram activity on all three test species, (Table 1 and 2, Figure 1 and 2). However, 10% was the most effective concentration, although this was evident only relatively soon after treatment. At picloram rates even lower than those tested, 10% may have remained the most effective ammonium sulfate concentration throughout the test period.

My results indicate that the greater effectiveness of high concentrations of ammonium sulfate may not always be evident because of such factors as the herbicide rate and the time after treatment of the observations. When added to 2,4-D Na salt, ammonium sulfate at 5% to 10% gave maximum enhancement of activity (Al'tergot and Kiselev 1963; Borodna et al. 1962; Makodzeba 1962), but ammonium sulfate at 1% or less appeared to be optimum for 2,4-D amine (Sexsmith 1953) and the sodium salt of dinitro-o-cresol (Crafts and Reiber 1945). While these reports are not necessarily directly comparable, factors such as herbicide rate and time of observation may contribute to conflicting reports on the most effective ammonium sulfate concentration for increasing herbicide activity.

The increase in picloram activity caused by ammonium sulfate can be attributed, at least in part, to an increase in picloram absorption by all three species, and also in strawberry guava, to an increase in the amount of picloram translocated out of the treated leaves (Table 3, 5 and 6). There was no evidence for or against a direct effect of this salt on picloram translocation in strawberry guava. The increase in the amount of picloram translocated clearly indicates that ammonium sulfate did not just increase picloram adsorption on the leaf surface or within the cuticle, but that ammonium sulfate must have increased picloram absorption into the leaves. Ammonium sulfate has not previously been reported to increase herbicide absorption. The greater

enhancement of picloram herbicidal activity with 10% ammonium sulfate compared with lower concentrations such as 0.5%, could not be accounted for by an increase in picloram absorption and translocation (Table 3), indicating that possibly other factors are also involved in the enhancement of activity.

Stomatal penetration could not have occurred in the two guava species because stomata are absent from the upper leaf surface of guava (Brun et al. 1961) and strawberry guava (nail polish replica examined under a microscope). The surface tension of an aqueous solution must be reduced for increased stomatal penetration to occur (Currier and Dybing 1959; Dybing and Currier 1959, 1961), and ammonium sulfate does not reduce the surface tension of aqueous solutions (Lange 1967). Thus even where stomata are present, as on the upper surface of bean leaves, ammonium sulfate would not be expected to aid stomatal penetration. Therefore in the measurement of the effect of ammonium sulfate on picloram absorption, cuticular penetration of picloram was involved.

Picloram absorption has been increased by reducing the pH of the treatment solution below 7 (Baur et al. 1971; Sargent and Blackman 1970). Ammonium sulfate at 0.5% reduced the pH of the ^{14}C-picloram solutions from pH 10 to 7.4, and the pH of the Tordon 22K solution used in measuring picloram absorption, from pH 7.8 to 6.2. In the latter case, the pH reduction could possibly result in increased absorption. However, further reduction of pH to 6.4 and 5.8 respectively,

by 10% ammonium sulfate, did not result in any additional increase in picloram absorption by either strawberry guava (Table 3) or beans (Table 6). Thus it appears unlikely that pH is the major factor in the enhancement of picloram absorption by ammonium sulfate.

The translocation of picloram in strawberry guava to the rapidly expanding leaves near the growing points, with or without ammonium sulfate added (Figure 3) is similar to that reported for other broad-leaved plants such as Canada thistle, soybean and bean (Hamill et al. 1972; Sharma and Vanden Born 1973; Sharma et al. 1971). The lack of an increase in picloram translocation following ammonium sulfate enhancement of absorption, as occurred in the guava (Table 4), is similar to results reported by Brady (1970). He found that ammonium nitrate increased the absorption of 2,4,5-T ester by four tree species, but an increase in the amount of 2,4,5-T translocated out of the treated leaves did not occur in three of the species. He suggested that there existed a limiting step in the translocation mechanism so that increased absorption did not result in increased translocation. A similar explanation may account for the failure of ammonium sulfate to increase picloram translocation in guava.

In summary, ammonium sulfate increased picloram activity on all three plant species tested and also enhanced picloram absorption in every case. The factors involved in ammonium sulfate enhanced absorption may lead to an understanding of the enhancement mechanism, and this aspect will be investigated

in the next chapter.

CHAPTER IV

AMMONIUM SULFATE ENHANCEMENT OF PICLORAM ABSORPTION BY DETACHED STRAWBERRY GUAVA LEAVES

Previous research (Chapter III) showed that ammonium sulfate increased picloram absorption in seedlings of strawberry guava (Psidium cattleianum Sabine), guava (Psidium guajava L.) and dwarf bean (Phaseolus vulgaris L. cv. Bountiful). This chapter deals with further investigations on the nature of the ammonium sulfate-enhanced absorption of picloram.

Ammonium sulfate reduces the pH of a picloram solution, and this factor alone may increase picloram absorption. The absorption of picloram by bean leaf discs and detached live oak leaves was increased by reducing the pH of the treatment solution below pH 7 (Baur et al. 1971; Sargent and Blackman 1970). Ammonium sulfate at 0.5% reduced the pH of picloram solutions used in absorption experiments (Chapter III), from pH 10 to 7.4 (^{14}C-picloram solution equivalent to Tordon 22K[1]), and pH 7.8 to 6.2 (Tordon 22K solution). In the latter case, the pH reduction could possibly result in increased absorption. However, further reduction of pH to 6.4 and 5.8 respectively, by 10% ammonium sulfate, did not result in any additional increase in picloram absorption. Thus it appears unlikely that pH is the major factor in the enhancement of

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[1]Trade name of Dow Chemical Company, Michigan.

picloram absorption by ammonium sulfate, but any role of this pH reduction in the ammonium sulfate enhancement effect, needs to be clearly established.

Picloram absorption was investigated in other studies which might indicate the nature of the ammonium sulfate enhancement mechanism. These were studies of the time-course of picloram absorption, the ion involved, the effect of picloram concentration, ammonium sulfate concentration, relative humidity, and leaf age.

Materials and Methods

Effect of ammonium sulfate on picloram absorption by detached leaves: Absorption of picloram was studied using leaves detached from seedlings of both guava species, grown in the glasshouse as described in Chapter III. Leaves were harvested just prior to use and placed across the edge of 5.5 cm plastic petri dishes, one leaf per dish, with the petiole resting in deionized water. Three leaves per treatment were used with each experiment repeated at least once.

Carboxyl-labelled ^{14}C-picloram acid (specific activity 4.13μCi/mg) was converted to the potassium salt using commercial formulation blank[1], as described previously (Chapter III). The ^{14}C-picloram K salt (hereinafter referred to as ^{14}C-picloram) was diluted with technical grade picloram K salt (76.8% a.i.) to a final total picloram concentration

[1]Supplied by Dow Chemical Company, Michigan.

of 1000 ppmw a.i. Ammonium sulfate was added to the appropriate treatment solutions. Each leaf was treated with two 5 μL droplets containing a total 0.025 μCi of ^{14}C-picloram. One droplet was placed on each side of the midrib and between the main lateral veins, on the upper leaf surface except as noted. The treated leaves were placed in a growth chamber at 30 C, 50 ± 5% RH and a light intensity of 23600 lux. At this humidity, the treatment droplets dried out in about 30 minutes. Each experiment was terminated at 12 hours, except as noted, by washing the residual picloram from the leaf surface with 50 ml of distilled water. The leaves were freeze-dried, oxidized, and the $^{14}CO_2$ collected in scintillation solution using the apparatus and methods of Peterson (1969) and Peterson et al. (1969). The samples were counted in a Packard liquid scintillation spectrometer.

To measure the time course of picloram absorption, strawberry guava leaves were treated as described above, and absorption measured at 1, 3, 6, 12, and 24 hours. All treated leaves were maintained in the light for the absorption period. A check was made on loss of ^{14}C by decarboxylation in the treated leaves. Unabsorbed picloram was washed from the treated leaf surfaces after 12 hours, and one set of leaves was returned to the growth chamber for a further 12 hour period to check for loss of ^{14}C.

To assess the influence of leaf age on the effect of ammonium sulfate on picloram absorption, leaves at three different ages were treated with picloram alone or plus

0.5% (w/v) ammonium sulfate. Young leaves were just fully expanded, light green, and very flexible. The intermediate aged leaves were more mature and taken from the second node down the stem. The old leaves, taken from the fourth or fifth node down the stem, were dark green, thick and not readily flexible.

The influence of picloram concentration on picloram absorption was measured by treating strawberry guava leaves with 0.025 μCi of ^{14}C-picloram in solutions of 250, 500, 1000, and 2000 ppmw of picloram alone or plus 0.5% (w/v) ammonium sulfate. To determine the effect of ammonium sulfate concentration on absorption, strawberry guava leaves were treated with picloram alone or plus ammonium sulfate at 0.01%, 0.1%, 0.5%, 1.0%, and 10% (w/v).

The effect of 0.5% and 10% (w/v) ammonium sulfate on picloram absorption was compared at a high relative humidity of 100% and the low relative humidity of 50 $\pm$ 5% normally used. To attain 100% humidity, the small petri dishes with treated leaves, were placed in a transparent plastic box after the treatment droplets had dried.

In experiments to measure the effect of the ammonium ion versus the sulfate ion, strawberry guava leaves were treated with picloram plus five ammonium salts and five sulfate salts, in addition to ammonium sulfate in each case. All salts were applied equimolar to 0.5% (w/v) ammonium sulfate, that is, 7.58×10^{-2}M ammonium or 3.79×10^{-2}M sulfate. The ammonium salts were nitrate, chloride, dibasic phosphate, carbonate

and molybdate. The sulfate salts of three univalent cations (lithium, sodium, and potassium), one divalent (magnesium) and one trivalent (aluminum) were used.

Influence of pH on the effect of ammonium sulfate on picloram absorption: The relationship between pH of the external solution and absorption of picloram was studied by adapting the method of Sargent and Blackman (1962) to use with detached strawberry guava leaves. A piece of plastic tube ca. 5 mm long and with an internal diameter of 6 mm was attached to the lower surface of each leaf with silicone grease. The treatment solution of 100 μL containing 250 ppmw of picloram (0.05 μCi ^{14}C-picloram) and 0.025 M of buffer, was confined within the tube. The buffers used were potassium biphthalate (pH 4 and 6.5) tris(hydroxymethyl)aminomethane (Tris)-maleate (Tris plus maleic anhydride) (pH 5.5 and 8), and potassium monobasic phosphate (pH 6 and 8). Each treated leaf was placed in a closed petri dish lined with a moistened filter paper, and with moistened cotton wool placed over the petiole. The dishes were placed in the growth chamber for the usual 12 hour treatment period. The effect of 0.5% (w/v) ammonium sulfate added to the ^{14}C-picloram solution buffered at pH 4 was measured with this method.

The effect of pH on the ammonium sulfate enhancement of picloram absorption was further studied by using ammonium dibasic phosphate and ammonium monobasic phosphate which have pK_a values of 7.20 and 2.15 respectively. The method

of applying two 5 μL droplets per leaf was used with ^{14}C-picloram treatment solutions of 1000 ppmw of picloram, plus ammonium dibasic and ammonium monobasic phosphate at 7.58×10^{-2}M ammonium, equimolar to 0.5% ammonium sulfate. Treatments with potassium dibasic phosphate and potassium monobasic phosphate, equimolar in dibasic and monobasic phosphate to the respective ammonium phosphates, were included as controls. Absorption by the upper and lower leaf surfaces was measured.

The effect of pH on picloram absorption alone or with ammonium sulfate added, was also measured from ^{14}C-picloram solutions adjusted to pH 4 and 7 with hydrochloric acid or sodium hydroxide.

In the statistical analysis of the data, the standard deviation (S.D.) of each mean was calculated. For six replicates, the standard deviation equals the 95% confidence limits, so that when the standard deviation limits of two means do not overlap, the means may be taken as significantly different.

Results

Effect of ammonium sulfate on picloram absorption by detached leaves: Ammonium sulfate enhanced picloram absorption by the upper and lower leaf surfaces of the detached leaves of both guava species (Table 7). The absorption of picloram by the upper and lower surfaces of strawberry guava

TABLE 7. -- Effect of $(NH_4)_2SO_4$ on the absorption of ^{14}C-picloram after application of 0.025μCi in two 5μL droplets to the upper and lower surfaces of leaves detached from the strawberry guava and guava seedlings.

Treatment	^{14}C-Picloram absorption leaf			
	Upper surface		Lower surface	
	(cpm)	(S.D.)	(cpm)	(S.D.)
Strawberry guava				
Picloram	106	70	104	34
Picloram + 0.5% $(NH_4)_2SO_4$	1536	546	472	135
Guava				
Picloram	55	20	161	21
Picloram + 0.5% $(NH_4)_2SO_4$	235	78	454	89

was equal. However ammonium sulfate enhanced absorption by the upper surface by approximately 14-fold compared with only a four-fold increase in absorption by the lower surface. With guava leaves, picloram absorption was greater through the lower surface, while the ammonium sulfate produced a similar enhancement on both surfaces.

Ammonium sulfate caused a rapid increase in picloram absorption compared with picloram alone (Figure 4). However absorption with or without ammonium sulfate present levelled off at 6 to 12 hours.

No major loss of ^{14}C was measured from leaves maintained in the growth chamber, for 12 hours after the initial 12 hour treatment with picloram alone or plus ammonium sulfate (Table 8).

Ammonium sulfate increased picloram absorption by leaves of all three ages (Table 9). Picloram absorption by strawberry guava leaves was not significantly affected by the external concentration of picloram between 250 and 2000 ppmw (Figure 5). The magnitude of the ammonium sulfate enhancement effect was about the same at all concentrations.

Concentrations of ammonium sulfate from 0.1% to 10% increased picloram absorption; 0.01% had no effect (Figure 6). Absorption was highest at an intermediate concentration range, such that greater than 0.1% but less than 10% ammonium sulfate was most effective.

FIGURE 4. -- Effect of $(NH_4)_2SO_4$ on the time course of absorption of ^{14}C-picloram after application of 0.025μCi in two 5μL droplets to detached strawberry guava leaves.

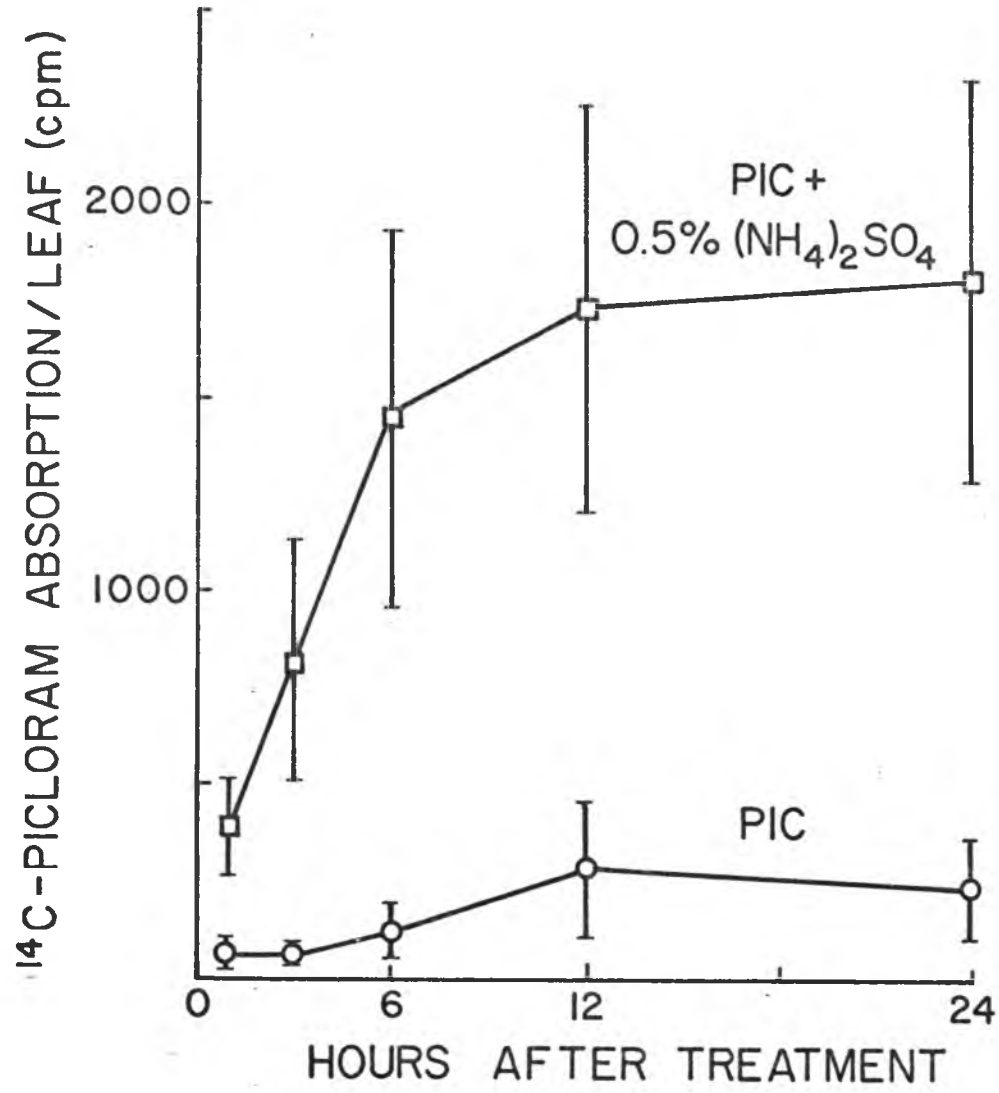

^{14}C-PICLORAM ABSORPTION/LEAF (cpm)
2000
1000
PIC +
0.5% $(NH_4)_2SO_4$
PIC
0
6
12
24
HOURS AFTER TREATMENT

TABLE 8. -- Amount of ^{14}C in detached strawberry guava leaves treated with ^{14}C-picloram alone or plus $(NH_4)_2SO_4$ for 12 hours and subsequently maintained in the growth chamber for a 12 hour period after washing the residual picloram from the leaf surface.

Treatment	^{14}C per leaf			
	at 12 hr		at 24 hr after washing at 12 hr	
	(cpm)	(S.D.)	(cpm)	(S.D.)
Picloram	104	25	171	55
Picloram + 0.5% $(NH_4)_2SO_4$	1490	375	1320	541

Increasing the relative humidity from 50% to 100% increased picloram absorption in all treatments (Table 10). The largest relative increase in absorption occurred with picloram alone, while both ammonium sulfate treatments were equally influenced by relative humidity.

Picloram absorption was increased by all the ammonium salts tested except ammonium carbonate and ammonium molybdate (Table 11). Ammonium sulfate was the only sulfate salt that increased absorption (Table 12).

TABLE 9. -- Influence of leaf age on the effect of $(NH_4)_2SO_4$ on the absorption of ^{14}C-picloram after application of 0.025μCi in two 5μL droplets to detached strawberry guava leaves.

Treatment	^{14}C-Picloram absorption leaf					
	Leaf age					
	Young		Intermediate		Old	
	(cpm)	(S.D.)	(cpm)	(S.D.)	(cpm)	(S.D.)
Picloram	106	43	277	164	84	25
Picloram + 0.5% $(NH_4)_2SO_4$	972	310	1744	348	901	89

FIGURE 5. -- Influence of picloram concentration on the effect of $(NH_4)_2SO_4$ on the absorption of ^{14}C-picloram after application of 0.025μCi in two 5μL droplets to detached strawberry guava leaves. Data are presented after multiplying cpm absorbed for the 500, 100, and 2000 ppmw concentration by two, four, and eight respectively, thus making the ratio of adjusted cpm absorbed to total picloram absorbed constant.

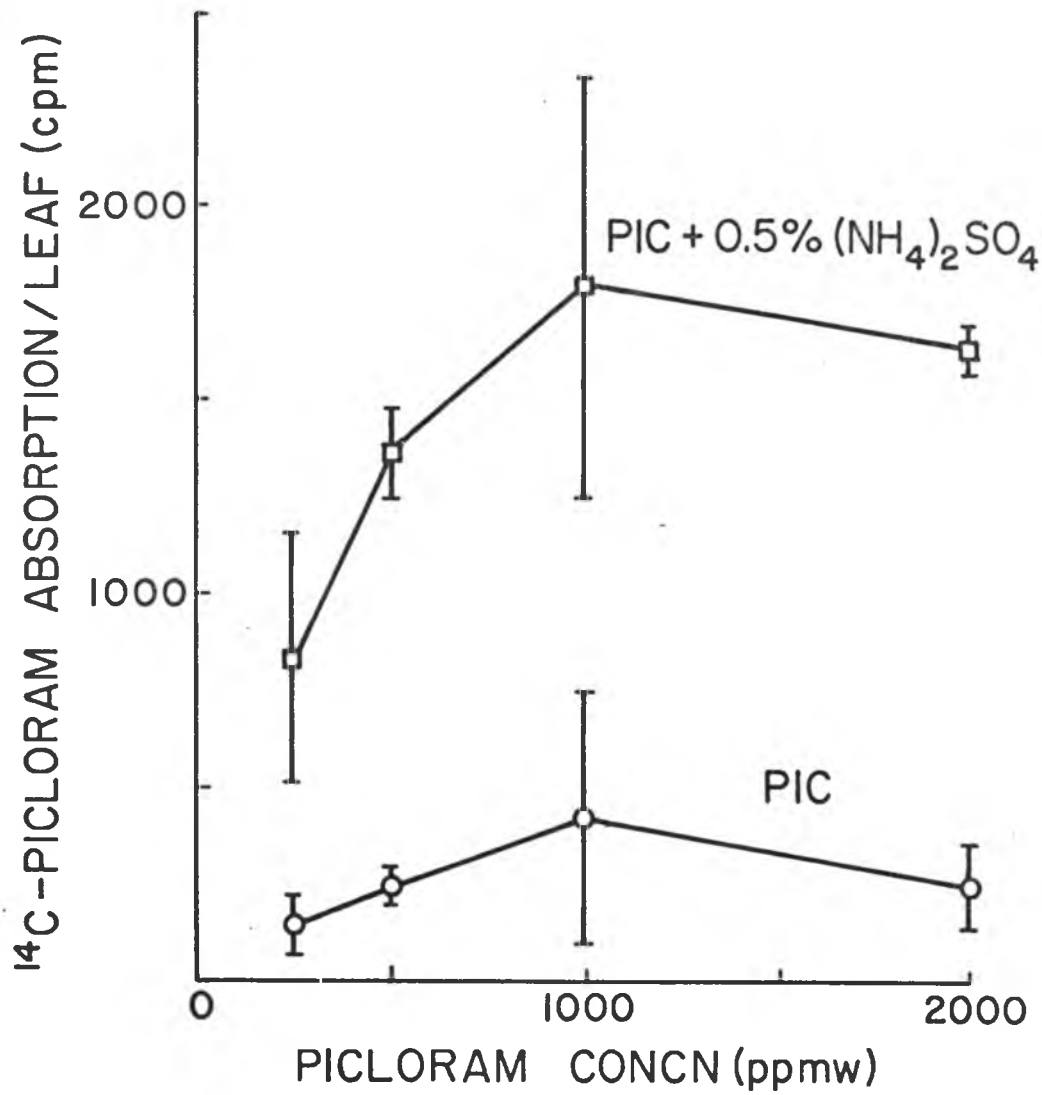

^{14}C-PICLORAM ABSORPTION/LEAF (cpm)
2000
1000
0
1000
2000
PICLORAM CONCN (ppmw)
PIC + 0.5% $(NH_4)_2SO_4$
PIC

FIGURE 6. -- Response of ^{14}C-picloram absorption by strawberry guava leaves to the concentration of added $(NH_4)_2SO_4$ after application of 0.025μCi of ^{14}C-picloram in two 5μL droplets.

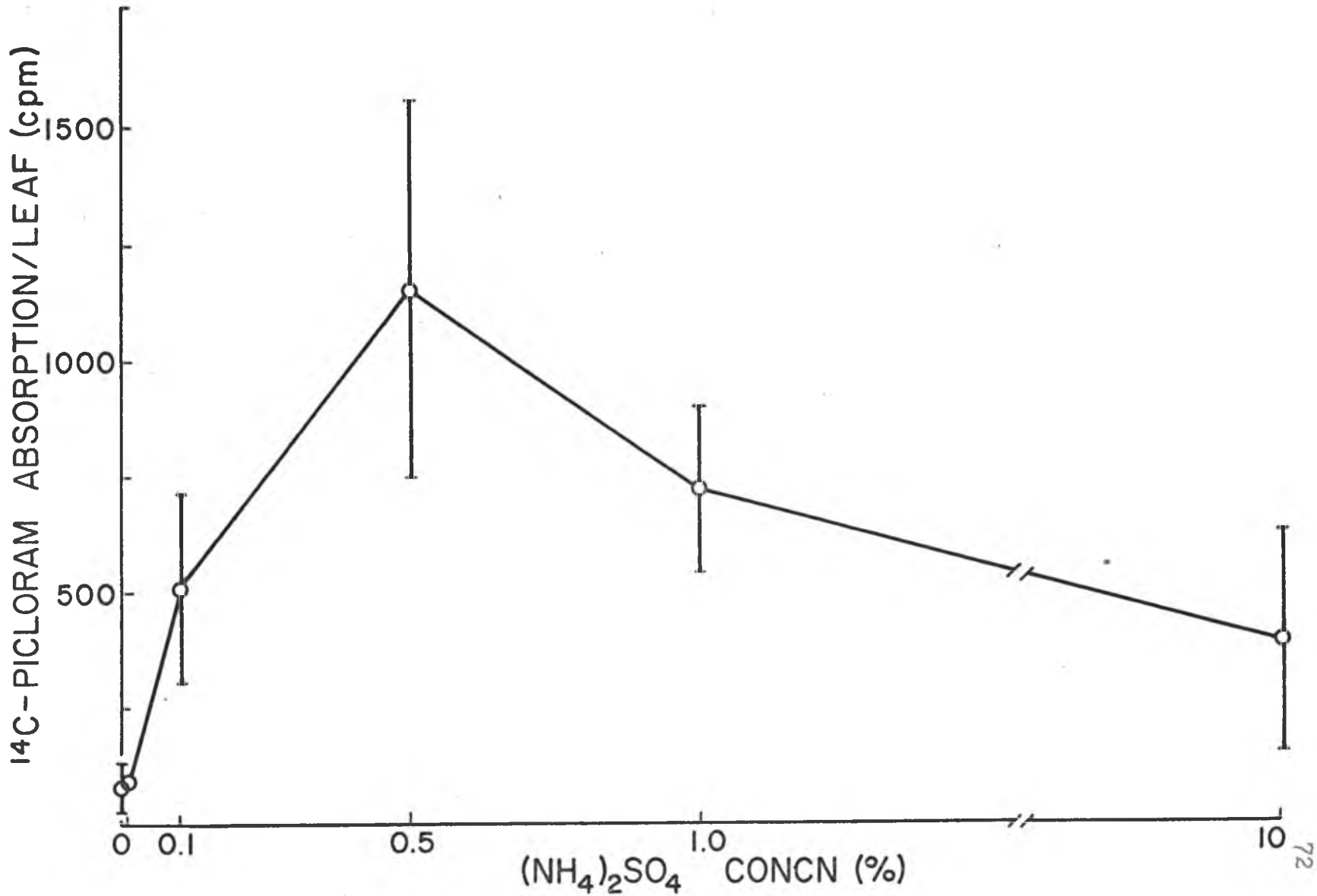

^{14}C-PICLORAM ABSORPTION/LEAF (cpm)
1500
1000
500
0
0.1
0.5
1.0
10
$(NH_4)_2SO_4$ CONCN (%)

TABLE 10. -- Influence of relative humidity on the effect of $(NH_4)_2SO_4$ on the absorption of ^{14}C-picloram after application of 0.025 μCi in two 5 μL droplets to detached strawberry guava leaves.

Treatment	^{14}C-Picloram absorption/leaf			
	Relative Humidity (%)			
	50		100	
	(cpm)	(S.D.)	(cpm)	(S.D.)
Picloram	35	11	212	69
Picloram + 0.5% $(NH_4)_2SO_4$	645	214	2030	891
Picloram + 10% $(NH_4)_2SO_4$	137	56	468	186

Influence of pH on the effect of ammonium sulfate on picloram absorption: Picloram absorption by the lower leaf surface increased markedly when pH of the external solution was reduced from pH 6 to 4 (Figure 7). Adjustment of the pH from 6 to 8 did not affect absorption. Values for absorption by the upper leaf surface were unusually erratic and are not presented.

Ammonium sulfate added to the picloram solution buffered at pH 4 did not increase absorption (Figure 7). The pH of the treatment solution dropped during the experiment when ammonium sulfate was added to the solutions buffered at pH 5.5 and above. Thus the effect of ammonium sulfate at pH

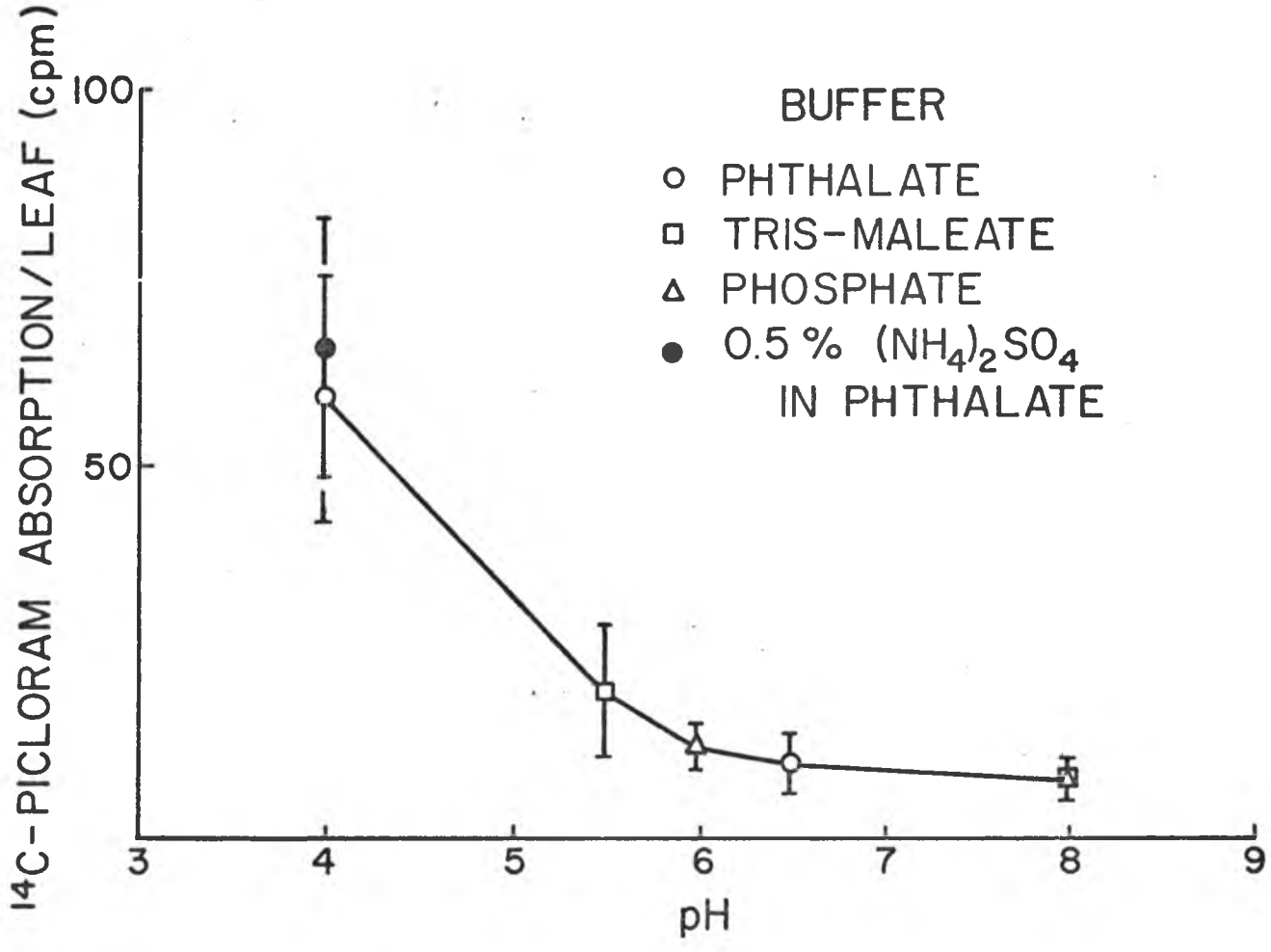
TABLE 11. -- Effect of six NH_4^+ salts at 7.58×10^{-2}M NH_4^+ (equivalent to 0.5% $(NH_4)_2SO_4$) on the absorption of ^{14}C-picloram after application of 0.025μCi in two 5μL droplets to detached strawberry guava leaves.

Treatment	^{14}C-Picloram absorption/leaf	
	(cpm)	(S.D.)
Picloram	319	152
Picloram + $(NH_4)_2SO_4$	1916	212
Picloram + NH_4NO_3	2333	941
Picloram + NH_4Cl	1252	389
Picloram + $(NH_4)_2HPO_4$	1848	546
Picloram + $(NH_4)_2CO_3$	431	235
Picloram + $(NH_4)_6Mo_7O_{24}.4H_2O$	479	172

TABLE 12. -- Effect of six $SO_4^=$ salts at 3.79×10^{-2}M $SO_4^=$ (equivalent to 0.5% $(NH_4)_2SO_4$) on the absorption of ^{14}C-picloram after application of 0.025 μCi in two 5 μL droplets to detached strawberry guava leaves.

Treatment	^{14}C-Picloram absorption/leaf	
	(cpm)	(S.D.)
Picloram	131	58
Picloram + Li_2SO_4	297	118
Picloram + Na_2SO_4	175	81
Picloram + K_2SO_4	207	123
Picloram + $(NH_4)_2SO_4$	1285	459
Picloram + $MgSO_4$	198	148
Picloram + $Al_2(SO_4)_3$	211	94

FIGURE 7. -- Effect of solution pH on picloram absorption by the lower surface of detached strawberry guava leaves, and the effect of $(NH_4)_2SO_4$ added at pH 4. ^{14}C-picloram (0.05μCi) applied in 100 L of solution contained on the leaf surface in a plastic tube.


^{14}C-PICLORAM ABSORPTION/LEAF (cpm)
100
50
3
4
5
6
7
8
9
pH
BUFFER
PHTHALATE
TRIS-MALEATE
PHOSPHATE
0.5 % $(NH_4)_2SO_4$ IN PHTHALATE

values other than pH 4 could not be measured with this method.

Ammonium dibasic phosphate increased picloram absorption as effectively as ammonium monobasic phosphate despite the difference in solution pH of 7.7 versus 4.6 (Table 13). A check on pH variation during the experiment was made with the method of Sargent and Blackman (1962) used above. The pH of the picloram plus ammonium dibasic phosphate solution dropped from 7.7 to 6.8, while the ammonium monobasic phosphate treatment solution remained at pH 4.6. Potassium dibasic phosphate had no effect on picloram absorption at pH 9.5, but absorption was increased by potassium monobasic phosphate at pH 4.6, to about one-half the absorption from the ammonium phosphate treatments (Table 13).

When the pH of the treatment solutions was adjusted by using hydrochloric acid or sodium hydroxide, a pH reduction from 7 to 4 had no effect on picloram absorption with or without ammonium sulfate added (Table 14).

TABLE 13.-- Effect of $(NH)_2HPO_4$ and $NH_4H_2PO_4$ on the absorption of ^{14}C-picloram after application of 0.025μCi in two 5μL droplets to detached strawberry guava leaves. $(NH_4)_2HPO_4$ and $NH_4H_2PO_4$ were added at 7.58 x 10^{-2}M NH_4^+ (equivalent to 0.5% $(NH_4)_2SO_4$) and K_2HPO_4 and KH_2PO_4 were added equimolar with the NH_4^+ phosphates for $HPO_4^=$ and $H_2PO_4^-$.

Treatment	pH of solution	^{14}C-Picloram absorption/leaf			
		Upper surface		Lower surface	
		(cpm)	(S.D.)	(cpm)	(S.D.)
Picloram	10.2	121	100	104	37
Picloram + $(NH_4)_2HPO_4$	7.7	850	456	410	173
Picloram + $NH_4H_2PO_4$	4.6	876	400	433	245
Picloram + K_2HPO_4	9.5	164	48	117	59
Picloram + KH_2PO_4	4.6	413	177	277	164

TABLE 14. -- Influence of solution pH on the effect of $(NH_4)_2SO_4$ on the absorption of ^{14}C-picloram after application of 0.025 μCi in two 5 μL droplets to detached strawberry guava leaves. pH adjusted with HCl or NaOH.

Treatment	^{14}C-Picloram absorption/leaf			
	pH of solution			
	4		7	
	(cpm)	(S.D.)	(cpm)	(S.D.)
Picloram	84	55	93	52
Picloram + 0.5% $(NH_4)_2SO_4$	835	404	705	267

Discussion

Picloram absorption through the lower leaf surface of guava was greater than through the upper surface (Table 7). This relative difference in absorption between leaf surfaces is in agreement with results of other workers for both picloram and other herbicides (Hull 1970; Sharma and Vanden Born 1970). In contrast, picloram absorption by strawberry guava leaves was equal from both surfaces, and with ammonium sulfate added, absorption was much greater through the upper surface.

Sargent and Blackman (1970) found a high loss of ^{14}C from ^{14}C-picloram absorbed in bean leaf discs. Caution is necessary in interpreting results when such a loss occurs. However, there was no major loss of ^{14}C from ^{14}C-picloram absorbed in strawberry guava leaves (Table 8).

Ammonium sulfate immediately increased the rate of picloram absorption by strawberry guava leaves (Figure 4), indicating that the mechanism of action of enhancement of picloram absorption probably involves a physical effect.

The enhancement of picloram absorption by ammonium sulfate was not appreciably affected by leaf age (Table 9). Leaves of intermediate and old age were used in the experiments in this chapter. There was an effect of leaf age on the absolute picloram absorption values, and this difference accounts for much of the variation in absorption values between experiments.

The effect of ammonium sulfate in increasing picloram

absorption is an effect that cannot be equalled by increasing picloram concentration (Figure 5). Picloram absorption by live oak leaves and bean leaf discs has been increased by increasing picloram concentration up to 242 ppmw (Baur et al. 1971) and 604 ppmw (Sargent and Blackman 1970) respectively, the maximum values tested in each case. Picloram absorption by strawberry guava leaves, with ammonium sulfate added, increased up to 500 ppmw and levelled off (Figure 5). There were no significant differences in the absorption of picloram applied alone at different concentrations.

Picloram absorption is not enhanced below a minimum concentration of ammonium sulfate (0.01% had no effect) (Figure 6). Absorption responds to increased ammonium sulfate concentration, so that a concentration range above 0.1%, but less than 10%, was most effective.

Increased picloram absorption at high relative humidity (Table 10) agrees with results reported by others (Hull 1970; Sharma and Vanden Born 1970). At high relative humidity, the dried out crust of ammonium sulfate on the leaf reabsorbed moisture. At 10% ammonium sulfate the re-moistened salt formed a droplet about as large as the original treatment droplet. On the leaves treated with 0.5% ammonium sulfate there was a relatively small amount of moisture covering only part of the treatment area, while there was no visible moisture on the leaves treated with picloram alone. This effect did not influence the relative picloram absorption difference between the 0.5% and 10% ammonium sulfate treatments.

The possibility arises that the deliquescence of ammonium sulfate is responsible for the enhancement effect. However at 50% relative humidity the salt deposit on the leaf surface appeared dry. Also, the enhancement effect at 50% relative humidity (35 cpm to 645 cpm) (Table 10) was greater than the increase in picloram absorption from increasing the relative humidity to 100% (35 cpm to 212 cpm), and even at 100% relative humidity there was a large enhancement effect (212 cpm to 2030 cpm). Thus the data indicate that the ammonium sulfate enhancement of picloram absorption is not due to increased moisture from the deliquescence of the salt.

The effect of different ammonium and sulfate salts on picloram absorption (Table 11 and 12) indicates that the ammonium ion is primarily responsible for the enhancement effect, although the action is modified by the anion present. My results agree with those of Horsfall and Moore (1962) who found that the activity of the sodium salt of naphthaleneacetic acod (NaNAA) was increased by a series of ammonium salts, but not the molybdate, borate, acetate, carbonate and bicarbonate. They suggested that only the ammonium salts of strong acids were effective, but this is not entirely true as ammonium citrate has increased picloram activity (Turner and Loader 1972) and ammonium borate has increased bean epicotyl bending by 2,4-D (Orgell and Weintraub 1957).

The data in Figure 7 show that picloram absorption by strawberry guava leaves increases at low pH value (below pH 6), but this differs somewhat from previous reports with

picloram. Absorption of picloram by live oak leaves and bean leaf discs increased as the pH was reduced below 7 (Baur et al. 1971; Sargent and Blackman 1970), and with the live oak leaves, the increase occurred only between pH 7 and 6.

The increase in picloram absorption with potassium monobasic phosphate added (pH 4.6) but not with potassium dibasic phosphate (pH 9.5) (Table 13), on both the upper and lower leaf surfaces, is presumably due solely to the effect of pH. In contrast, ammonium dibasic and monobasic phosphates equally increased absorption despite the differences in treatment solution pH of 7.7 versus 4.6. Thus the reduction of the pH of picloram solutions by ammonium is not necessary for the enhancement of picloram absorption.

Adjusting the pH down to 4 using hydrochloric acid in treatment solutions applied in a small volume to the leaf surface, did not increase picloram absorption (Table 14). This appears to be an inadequate method to study the effect of pH on absorption. Sargent and Blackman (1970) indicated that if pH values in phosphate buffered solutions are initially low, the pH increases when the solution is maintained on the leaf surface in an absorption experiment. Thus an even more marked increase in pH resulting in no pH effect on absorption, could be expected when a small volume of solution is applied without a buffer designed to maintain the pH.

As noted in Chapter III the measurement of the effect of ammonium sulfate on picloram absorption involves cuticular penetration. The mechanism of action by which ammonium

sulfate enhances absorption has not been determined. However the enhancement of picloram absorption is not necessarily due to lower solution pH values (Table 13), nor to any deliquescent effect of ammonium sulfate, and cannot be equalled by increasing picloram concentration (Figure 5). Any advantage from reduced surface tension is not involved, as ammonium sulfate does not reduce the surface tension of an aqueous solution (Lange 1967). The elimination of these as factors, and the fact that the effect of ammonium sulfate on absorption is immediate (Figure 4), suggests that ammonium sulfate has a direct physical effect in the absorption pathway.

Cuticular absorption of a herbicide involves movement through the cuticle and the epidermal cell wall (Franke 1967; Foy 1964). Picloram is phloem-translocated (Hamill et al. 1972; Sharma and Vanden Born 1973; Sharma et al. 1971), so it must also cross the plasmalemma. The cuticle is the first barrier to absorption, and studies with isolated cuticle show that it is a major barrier to penetration of polar compounds (Darlington and Cirulis 1963; Goodman and Addy 1963; Orgell 1957; Yamada et al. 1964a). Thus the cuticle is a likely site for the enhancement effect of ammonium sulfate on picloram absorption.

A mechanism by which ammonium sulfate could act in the cuticle can be proposed. Cutin has a negative charge at neutral or high pH due to dissociation of the polar groups (Crafts and Foy 1962; Franke 1967; Orgell 1957). At the pH values of the picloram solutions used, pH 5.8 and higher

(Chapter III), picloram is dissociated (Sargent and Blackman 1960), so absorption involves the picloram anion. Electrostatic repulsion between the negative charge of the cuticle and the penetrating anion could slow foliar absorption. It is proposed that the ammonium cation reduces the cuticular negative charge thus allowing the picloram anions to penetrate through the cuticle more readily. The finding that the ammonium ion appears to be responsible for the enhancement (Table 11 and 12), and that the salt acts immediately to increase absorption (Figure 4) is consistent with the hypothesis.

A proposal to account for increased herbicide absorption at low pH, attributes the increase to reduced ionization of the polar groups in the cuticle (van Overbeek 1956). This mechanism is similar to the proposed mechanism by which ammonium sulfate increases absorption. If the mechanisms are similar, then ammonium sulfate should not produce any increase in absorption at low pH. At pH 4 in this study, ammonium sulfate did not increase picloram absorption (Figure 7), thus supporting the hypothesis.

CHAPTER V

SUMMARY AND CONCLUSIONS

A possible approach to increasing herbicidal control of guava (Psidium guajava L.) and strawberry guava (Psidium cattleianum Sabine) is to increase herbicide activity with an inorganic salt adjuvant. Ammonium sulfate increased picloram activity on both guava species and dwarf beans (Phaseolus vulgaris L. cv. Bountiful), in agreement with the results of Turner and Loader (1972) and other reports of ammonium sulfate enhancement of herbicide activity (Al'tergot and Kiselev 1963; Borodna et al. 1962; Chesalin 1962; Ladonin 1958, 1961; Likholat 1962; Lobarchuk 1962; Makodzeba 1962; Sexsmith 1953; Shcheglov 1962; Wort 1962; Zambin 1962). Ammonium sulfate at a concentration of 0.75% to 1.0% was as effective as higher concentrations, except shortly after treatment when picloram activity was increased by increasing salt concentration up to 10%. The evidence suggests that at picloram rates lower than those tested, the greater effectiveness of 10% would remain evident.

Ammonium sulfate increased picloram absorption by the two guava species and dwarf beans. An ammonium sulfate-induced increase in herbicide absorption has not previously been reported. The greater effectiveness of 10% ammonium sulfate on picloram activity compared with 0.5% could not be explained by increased absorption or translocation of picloram. This suggests that the effect of ammonium sulfate on

picloram absorption does not completely account for increased picloram activity. With ammonium sulfate added to the picloram solution the amount of picloram which moved out of the treated leaves was increased in strawberry guava but not in guava. Since increased picloram absorption in guava was not reflected in increased translocation, there may be a limiting step in picloram translocation in guava, which does not occur in strawberry guava.

In studies with detached leaves, ^{14}C-picloram absorption through the upper and lower leaf surfaces of strawberry guava was equal, and with ammonium sulfate added, absorption was much greater through the upper surface. In guava leaves picloram absorption was greater through the lower surface, and this agrees with results of other workers for both picloram and other herbicides (Hull 1970; Sharma and Vanden Born 1970). The enhancement of picloram absorption in detached strawberry guava required a minimum concentration of ammonium sulfate (0.01% had no effect), and responded to increased salt concentration, so that a concentration range above 0.1% but less than 10%, was most effective. Increased ^{14}C-picloram absorption resulted from adding the ammonium salts, sulfate, nitrate, chloride or dibasic phosphate, but not from ammonium carbonate or ammonium molybdenate. Ammonium sulfate was the only sulfate salt found to increase ^{14}C-picloram absorption, thus the enhancement effect appears to be primarily due to the ammonium ion although its action is modified by the anion present.

The enhancement effect of ammonium sulfate could not be equalled by increasing the picloram concentration, and absorption results at low and high humidity indicate that the effect is not due to any deliquescent effect of ammonium sulfate. Ammonium dibasic and monobasic phosphates at pH values of 7.7 and 4.6 respectively, were equally effective in increasing picloram absorption, so a reduction in solution pH is not necessary for the enhancement effect. Ammonium sulfate does not reduce the surface tension of aqueous solutions (Lange 1967), so any advantage of reduced surface tension is not involved. Ammonium sulfate acted immediately to increase picloram absorption, so this, along with the elimination of the above factors as possible mechanisms of action, suggests that ammonium sulfate has a direct physical effect in the absorption pathway. The cuticle is the first barrier to absorption, and studies with isolated cuticle show it is a major barrier to penetration of polar compounds (Darlington and Cirulis 1963; Goodman and Addy 1963; Orgell 1957; Yamada et al. 1964a), so the cuticle is a likely site for the action of ammonium sulfate on picloram absorption.

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